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Abstract
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Secreted Frizzled-Related Protein 4 Reduces Insulin Secretion and Is Overexpressed in Type 2 Diabetes

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SUMMARY

A plethora of candidate genes have been identified for complex polygenic disorders, but the underlying disease mechanisms remain largely unknown. We explored the pathophysiology of type 2 diabetes (T2D) by analyzing global gene expression in human pancreatic islets. A group of coexpressed genes (module), enriched for interleukin-1-related genes, was associated with T2D and reduced insulin secretion. One of the module genes that was highly overexpressed in islets from T2D patients is SFRP4, which encodes secreted frizzled-related protein 4. SFRP4 expression correlated with inflammatory markers, and its release from islets was stimulated by interleukin-1β. Elevated systemic SFRP4 caused reduced glucose tolerance through decreased islet expression of Ca²⁺ channels and suppressed insulin exocytosis. SFRP4 thus provides a link between islet inflammation and impaired insulin secretion. Moreover, the protein was increased in serum from T2D patients several years before the diagnosis, suggesting that SFRP4 could be a potential biomarker for islet dysfunction in T2D.

INTRODUCTION

Large-scale gene expression data hold great promise to provide pathophysiological insights for a range of diseases, but there are several limitations with our current way of analyzing such information (Lander, 2011; Rosengren et al., 2010; Saxena et al., 2007; Schadt, 2009; Sladek et al., 2007; Voight et al., 2010; Zeggini et al., 2008). Network models, in which genes are represented as nodes and their connections as edges, have been proposed as a useful framework for studying complex biological data (Barabasi and Albert, 1999; Barabási et al., 2011; Ravasz et al., 2002; Schadt, 2009; Taneera et al., 2012). To take full advantage of those models, it is essential to analyze disease-relevant tissues and to combine bioinformatics with mechanistic validations. Using type 2 diabetes (T2D) as an example of a complex polygenic disorder, we have explored whether coexpression networks in human pancreatic islets could be used to identify disease genes.

RESULTS

Gene Coexpression Analysis Identifies a Module that Is Associated with T2D, Elevated HbA1c, and Reduced Insulin Secretion

Impaired insulin secretion is a cardinal feature of T2D, typically combined with insulin resistance, but the underlying mechanisms are largely unknown. We obtained global microarray expression data from islets from 48 human donors, of which 10 had T2D (see Table S1 available online), and investigated the gene expression topology with the weighted gene coexpression network analysis framework developed by Zhang and Horvath (Zhang and Horvath, 2005) (see the Supplemental Experimental Procedures). The connectivity, reflecting coexpression, was calculated for all pairs of gene expression traits. The resulting coexpression network exhibited the characteristic scale-free properties (R² > 0.9; Table S2) of biological networks (Ravasz et al., 2002). Next, the topological overlap, as a measure of relative gene interconnectedness, was computed and used to identify 17 gene modules (Figure 1A).

Rather than analyzing each gene expression trait individually, the first principal component of the gene expression traits of each module (the “module eigengene”) was used. We identified an eigengene representing a module with 174 genes (Table S3), which had higher values in islets from diabetic donors (p = 0.010; Table S4) and was correlated with HbA1c, reflecting long-term blood glucose (p = 0.010 in all donors; n.s. when analyzing only nondiabetic [ND] donors) and reduced glucose-stimulated...
insulin secretion (p = 0.0047 in all donors; n.s. in ND). The corre-
sponding module was enriched for interleukin-1 (IL-1)-binding
 genes (43.3-fold enrichment; p = 0.026) and IL-1 receptor activity
(61.8-fold enrichment; p = 0.0079) as well as RNA-processing
genes. No other module eigengene was significantly associated
with T2D (Table S4; p values are nominal without correction for
multiple testing).

We explored the possibility of using topological information to
prioritize genes in the T2D-associated module. The total connec-
tivity of a gene (the degree \( k \)) is a central parameter, as scale-free
networks are characterized by a few high-degree hubs, which
connect a large number of peripheral nodes (Albert et al.,
2000; Carter et al., 2004; Ravasz et al., 2002).

For each gene in the T2D module, we analyzed the con-
nectivity within (\( k_{in} \)) and outside (\( k_{out} \)) the module (Zhang and
Horvath, 2005). There was a significant correlation between \( k_{in} \),
but not \( k_{out} \), and gene expression association with diabetes status
(Figures 1B–1D). We therefore selected the most connected
genes in the module for further analyses. Of these, the expression of SFRP4, ASAM, and FBLN1 was most highly asso-
ciated with T2D. IL18R1, IL1RL1, and SFRP4 correlated most
strongly with HbA1c and SFRP4, IL18R1, and ABCE1 with insulin
secretion. SFRP4 was among the top three hub genes associ-
cated with all T2D traits (Figures 1E–1G).

The network analysis was replicated in another data set using
human islet microarrays from 29 donors, 10 of which had T2D
(Table S5). The coexpression network generated from these
data also exhibited scale-free properties (\( R^2 > 0.8 \); Table S6),
and 20 gene modules were identified (Table S7). Statistical
power was lower in this smaller data set, and no module eigengene
was significantly associated with T2D. However, an eigengene
representing a module with 123 genes (Table S8) was
nominally associated with HbA1c (p = 0.034, all 29 donors) and
showed a tendency for higher values in diabetic donors (p = 0.072).
Interestingly, the module contains SFRP4, as well as a high degree of shared genes with the T2D-associated module
identified in the 48 donors initially analyzed (p = 1.9E-11 using
Fisher’s exact test), including IL-1 receptors (85.9-fold enrich-
ment). There was an association between \( k_{in} \) and gene ex-
pression association with diabetes status (p = 0.0003) also in
the replication set, and SFRP4 was among the most highly con-
nected genes in the module.
**SFRP4** encodes secreted frizzled-related protein 4, which is a 40 kDa protein with an N-terminal cysteine-rich domain with sequence similarity with frizzled (Fz) receptors and a C-terminal netrin-like domain that has been suggested to interact with extracellular matrix proteins (Bovolenta et al., 2008; Ratner et al., 1997). SFRP4 is an extracellular regulator of the Wingless (Wnt) pathway. Interestingly, several T2D-associated genetic variants locate to genes in the Wnt pathway (Saxena et al., 2007; Sladek et al., 2007; Voight et al., 2010; Zeggini et al., 2008), including the Wnt effector TCF7L2 (da Silva Xavier et al., 2009; Grant et al., 2006). SFRP4 is involved in tissue development and cancer, as well as phosphate metabolism (Berndt et al., 2003; Bovolenta et al., 2008; Heller et al., 2002), but to date it has not been implicated in T2D.

**SFRP4 Reduces Insulin Secretion through Decreased Ca^{2+} Influx and Insulin Exocytosis in Pancreatic β Cells**

We studied the effect of SFRP4 on insulin secretion by exposing isolated mouse pancreatic islets to recombinant SFRP4 for 24 hr followed by 1 hr stimulation with 16.7 mM glucose. The peptide dose-dependently reduced glucose-stimulated insulin secretion with maximal inhibition observed at 30 nM (Figures 2A and S1A); insulin content or β cell viability were not affected (Figures S1B–S1D). A comparable inhibitory effect of 24 hr treatment with SFRP4 on glucose-stimulated insulin secretion was also observed in human islets (Figure 2B). SFRP4 did not affect basal insulin secretion (measured at 2.8 mM glucose) in mouse (Figure 2A) or human islets (Figure 2B), and there was no association between basal insulin release and SFRP4 expression (p = 0.2). In both human and mouse islets, insulin secretion was unaffected by acute exposure to SFRP4 (Figures S1E and S1F).

Pancreatic β cells release insulin through Ca^{2+}-dependent exocytosis, which can be monitored as increases in cell capacitance (Rorsman and Renström, 2003). Pretreatment with SFRP4 decreased the exocytotic responses to a train of ten depolarizations (applied to simulate glucose-induced electrical activity) in both human (Figure 2C) and mouse β cells (Figure S1G) relative to nontreated control cells. This was paralleled by a reduction of the integrated Ca^{2+} current (that reports the cumulative Ca^{2+} entry) (Figures 2D and S1H). However, there was no change in the Ca^{2+} sensitivity of the exocytotic process (defined as the capacitance increase divided by the integrated Ca^{2+} current; Figure S1I), which suggests that the exocytosis machinery was intact. Conversely, siRNA-mediated silencing of SFRP4 in dispersed mouse β cells (63% ± 2% mRNA knockdown; n = 3) increased Ca^{2+} influx by 65% and exocytosis by 42% (Figure 2E) and was paralleled by a 30% reduction of accumulated SFRP4 secreted into the medium following transfection. The siRNA treatment had no effect on insulin secretion from intact mouse islets (Figure S1J), putatively due to poor transfection efficiency in the islet core, which confounds the whole-islet experiments. To specifically study the effects of SFRP4 originating from β cells, we silenced SFRP4 in the clonal β cell line INS832/13 (76% ± 6% mRNA knockdown), which enhanced glucose-stimulated insulin release by 25% (p = 0.029, one-tailed; Figures S1K and S1L).

Next, electrical activity was assessed using current-clamp recordings of mouse islet cell clusters treated with or without SFRP4. The electrical activity consisted of depolarized plateaux on which action potentials were superimposed, interrupted by periods of repolarization. There was a tendency for lower action potential amplitude and frequency in SFRP4-treated cell clusters compared with control cells (Figure S2), while the plateau potential did not differ between the conditions (~43 ± 1 mV without and ~42 ± 1 mV with SFRP4). To specifically study the effect of SFRP4 on glucose-induced changes in intracellular Ca^{2+} ([Ca^{2+}]i) we conducted ratiometric measurements with Fura-2. These experiments showed that the peak increase in [Ca^{2+}]i elicited by an elevation of glucose from 2.8 to 16.7 mM was reduced by ~65% in islets treated with SFRP4 for 24 hr (p = 0.025; Figures 2F and 2G). Furthermore, SFRP4-treated islets exhibited reduced insulin secretion both in response to 200 μM tolbutamide (which closes the ATP-sensitive K+ channels [K_{ATP} channels]) and high (50 mM) K+ (leading to membrane depolarization) (Figure 2A). Taken together, the data suggest that SFRP4 inhibits insulin secretion at a step distal to K_{ATP} channel closure and cell depolarization through suppressed Ca^{2+} influx.

**SFRP4 Affects Wnt Signaling and L-Type and P/Q-Type Ca^{2+} Channels**

SFRP4 has variably been reported to activate or inhibit Wnt signaling in different tissues (Carmon and Loose, 2008; Feng et al., 2009; Gelebart et al., 2008; Lee et al., 2008; Park et al., 2009; Suzuki et al., 2004). The Wnt pathway is activated via G_{i/o}-coupled frizzled receptors (Liu et al., 2001), and inhibition of G_{i/o} proteins by pertussis toxin abolished SFRP4-mediated suppression of insulin secretion (Figure 3A). Furthermore, the ratio of unphosphorylated versus total β-catenin was elevated in SFRP4-treated mouse islets, suggestive of canonical Wnt activation (Figure 3B). Unphosphorylated β-catenin translocates to the nucleus where it affects TCF/LEF transcription factors (Grant et al., 2006; Gelebart et al., 2008; Lee et al., 2008; Suzuki et al., 2004). The Wnt pathway is activated via Wnt/β-catenin translocation (Figure 3A), confirming the stimulatory effect on Wnt signaling. Noncanonical Wnt pathways were unaffected (Figures S3A–S3E). SFRP4 inhibition of β cell exocytosis was not influenced by cotreatment with canonical (Carmon and Loose, 2008) or noncanonical Wnt proteins (Ma and Wang, 2007) (Figure S3F).

TGF/LEF activation has been shown to repress Ca^{2+} channel expression (Wisniewska et al., 2010), and we investigated whether SFRP4-induced suppression of insulin release involved altered expression of Ca^{2+} channels. Notably, both L-type (Ca_{L}1.2 and Ca_{L}1.3) and P/Q-type (Ca_{PQ}2.1) Ca^{2+} channels were downregulated by SFRP4 in human β cells (Figure S4A). T-type Ca^{2+} channels (Ca_{T}3.2) were unaffected. We next analyzed the effect of the L-type Ca^{2+} channel blocker isradipine and the P/Q-type blocker ω-agatoxin IVA (Braun et al., 2008) on the current-voltage (I-V) relationship in human β cells incubated with or without SFRP4 for 24 hr. SFRP4-treated cells displayed a pronounced reduction of the peak current (~55% at 0 mV; p = 3.5E–5; Figure 3D). Interestingly, while isradipine suppressed the current in control cells (p = 0.028 at 0 mV), it was without effect in SFRP4-treated β cells (Figure 3D). Likewise, ω-agatoxin IVA decreased the peak current in control cells (p = 0.001 at 0 mV), but was largely ineffective in SFRP4-treated β cells, except for a tendency (p = 0.3) for a suppression...
Figure 2. Effects of SFRP4 on Insulin Secretion and β Cell Exocytosis

(A) Insulin secretion in response to 1 hr incubations with 2.8 mM glucose, 16.7 mM glucose, 2.8 mM glucose plus 50 mM K+, or 8.3 mM glucose plus 200 μM tolbutamide in mouse islets that were cultured with or without 30 nM SFRP4 for 24 hr prior to experiments. SFRP4 was not present during the 1 hr incubations. Data are from 6–12 experiments per group. p values are from unpaired comparisons.

(B) Insulin secretion in response to 1 hr incubations with 2.8 or 16.7 mM glucose in human islets cultured in the absence or presence of 30 nM SFRP4 for 24 hr. Data are from 5–8 experiments per group from each of three donors at 2.8 mM glucose, and data from 4–12 experiments per group from each of 17 donors at 16.7 mM glucose. p values are from paired comparisons for each donor.

(C) Increase in cell capacitance (ΔC), reflecting exocytosis, evoked by a train of ten 500 ms depolarizations from −70 to 0 mV applied to a human β cell to simulate glucose-induced electrical activity. Histogram shows total capacitance increase (ΔΔC) in human β cells preincubated without or with 30 nM SFRP4 for 24 hr. SFRP4 was not present in the extracellular solution during the recordings. Data are from 3–11 experiments per donor from 16 donors with paired comparisons.

(D) The Ca2+ current (I) in response to the first depolarization of the train. The bars denote the integrated Ca2+ current (charge) in human β cells preincubated without or with SFRP4. Data are from same cells as in (C).

(E) Left histogram shows average total capacitance increase in response to a train stimulus in mouse β cells treated with control siRNA or siRNA against SFRP4. Right bars show the mean integrated Ca2+ current (charge) in response to the first depolarization. Data are from 17 and 39 cells, respectively. p values are from unpaired one-sided tests.

(F) Representative recordings of Fura-2 fluorescence at 340 versus 380 nm (ΔF340/380 from the average baseline) in mouse islets that had been incubated for 24 hr in the absence or presence of 30 nM SFRP4. The extracellular glucose concentration was changed from 2.8 to 16.7 mM during the experiment as indicated. Sampling rate is 0.2 Hz.

(G) The histogram shows the mean ΔF340/380 peak at high glucose in control (n = 5) and SFRP4-treated islets (n = 5). Data are mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001.
at 0 mV (~25% compared with the ~55% reduction in control cells). These findings suggest that both L-type and P/Q-type Ca2+ channels are suppressed by SFRP4. Indeed, the expression of SFRP4 in human islets was negatively correlated with CACNA1A (CaV2.1) (p = 0.008; r = −0.376) and CACNA1D (CaV1.3) mRNA (p = 0.003; r = −0.426).
SFRP4 Expression and Release Are Stimulated by Interleukin-1β

The regulation of SFRP4 production and release has not been characterized. Immunohistochemistry demonstrated SFRP4 in both α and β cells without preferential accumulation in the secretory granules (Figures 3F and S4B). Stimulation for 1 hr with 16.7 mM glucose or 50 mM K+ to elicit depolarization-induced exocytosis did not affect SFRP4 release, indicating that SFRP4 is released in a constitutive fashion rather than cosecreted with insulin (Figures S4C and S4D).

There was no significant association between known T2D gene variants and SFRP4 expression (Table S9). Since the T2D module containing SFRP4 was enriched for IL-1-related genes, we hypothesized that SFRP4 might be an inflammatory mediator. Interestingly, human islets incubated with IL-1β displayed a 1.8-fold elevation of SFRP4 mRNA (Figure 3G) and a 3-fold higher secretion of the protein into the medium (Figure 3H). Moreover, 24 hr treatment of human islets with 100 μM YVAD–CMK, an inhibitor of IL-1β convertase that cleaves and activates the IL-1β precursor (Samad et al., 2001), or recombinant human IL-1 receptor antagonist (500 ng/ml) reduced SFRP4 secretion (Figures S4E and S4F). SFRP4 expression correlated with islet content of IL-6 (p = 5E–5; r = 0.58) and IL-8 (p = 0.001; r = 0.52). By contrast, IL-1β, IL-6, and IL-8 expression was not affected by SFRP4 exposure (Figures 3G, S4G, and S4H), which suggests that SFRP4 acts downstream of IL-1β to reduce insulin secretion. Indeed, analysis of transcription factor binding sites near SFRP4 (see Supplemental Experimental Procedures) demonstrated several putative binding sites for NF-κB, which is elevated by IL-1β (Figure 3G).

Moreover, the inhibitory effect of IL-1β on insulin secretion was attenuated (p = 0.001) in INS832/13 cells transfected with siRNA against SFRP4 compared with cells treated with an inactive control siRNA (Figure S4I). Due to incomplete SFRP4 knockdown (76% ± 6%) it is, however, difficult to estimate the exact extent by which SFRP4 contributes to the IL-1β effect.

SFRP4 Injections In Vivo Reduce Insulin Secretion in Mice, and SFRP4 Is Elevated in Serum from T2D Patients

Next, mice were injected three times over 24 hr with SFRP4, which resulted in glucose intolerance and reduced insulin secretion (Figure 4A). Interestingly, isolated islets from SFRP4-treated mice displayed decreased glucose-induced insulin secretion as well as suppressed β cell Ca2+ influx and exocytosis (Figures 4B and 4C). This demonstrates that elevated SFRP4 in vivo induces sustained inhibitory effects on β cell function.

Finally, we investigated SFRP4 concentration in serum from 88 individuals (Groop et al., 1996) who were analyzed at three different visits (median interval 3 years) (Table S10). Serum levels of SFRP4 were significantly elevated in T2D patients (p = 0.018, age-, sex- and BMI-corrected; n = 23) compared with ND subjects (n = 51) (Figure 4D). Interestingly, ND individuals who later developed T2D (“converters”; n = 14) had significantly higher SFRP4 already at the initial visits and prior to presentation of the disease (p = 0.046) (Figure 4E). Among ND individuals with SFRP4 levels below the median (23.7 ng/ml), 9% (3 out of 35 subjects) developed T2D at a later stage, while 37% (11 out of 30) of those with SFRP4 above the median developed the disease (Table S11). The odds ratio (OR) was 5.35 (95% CI: 1.29–22.2; p = 0.021, age-, sex- and BMI-corrected).

These analyses were replicated in an extended cohort with 69 converters and 69 matched controls, in which serum SFRP4 was analyzed and an oral glucose tolerance test (OGTT) conducted at two consecutive visits (median interval 4 years [range 1–15 years]) (Table S12). The converters had been classified as ND at the first visit but were diagnosed with T2D at the second visit. In ND individuals, SFRP4 was associated with higher fasting glucose (p = 0.004; beta = 0.142), reduced insulin sensitivity index (p = 0.002; beta = −0.176), and lower disposition index (insulin secretion adjusted for insulin sensitivity; p = 0.029; beta = −0.186; Table S13). Serum concentration was not affected by BMI, sex, patient age, or sample age (Table S13). Supporting the results from the initial analysis, SFRP4 was significantly elevated in T2D individuals by the time of diagnosis (the second visit) compared with controls (39% increase; one-tailed p = 0.044 and beta = 0.164 with age, sex, and BMI correction; Figure 4F). Interestingly, and further corroborating the initial findings, converters had increased SFRP4 already at their first visit, prior to clinical diagnosis (37% increase compared with controls; one-tailed p = 0.034 and beta = 0.163 with correction for age, sex, and BMI). The p values were 0.12 and 0.07 (one-tailed), respectively, if adjusting for fasting glucose and glucose at 2 hr during the OGTT, which suggests that the effect is largely driven by the association between SFRP4 and glucose levels. We next studied the usefulness of categorizing individuals into quartiles based on serum SFRP4 at the first visit. As shown in Figures 4G and 4H, individuals with SFRP4 in the upper quartile were at particular risk of developing T2D (p = 0.005; OR 3.32 [95% CI: 1.34–8.24]) compared with subjects in quartiles 1–3. The risk elevation was significant even after correction for fasting glucose (p = 0.018; OR = 2.89 [95% CI: 1.07–7.85]) or glucose at 2 hr during the OGTT (p = 0.022; OR = 3.05 [95% CI: 1.04–9.0]). Further study is needed to assess if SFRP4 can be used as a biomarker for T2D risk independent of glucose.

DISCUSSION

The study identifies SFRP4 as a molecular link between islet inflammation and defective insulin secretion. It also demonstrates the utility of modular analyses of biological data to find disease genes that are not obvious candidates from genetic association studies, although it is difficult to establish causality from expression studies with a restricted number of subjects. SFRP4 affects Wnt signaling, which influences a wide scope of genes, here reflected by the suppression of two different voltage-gated Ca2+ channels that in turn lead to reduced insulin exocytosis. Indeed, the main effect of SFRP4 in β cells was the reduction of insulin secretion stimulated by glucose and other secretagogues, while insulin content and β cell viability were unaltered. It is of interest that the Wnt pathway has recently been implicated in the pathogenesis of T2D from the identification of several T2D-associated DNA variants that locate near genes in the Wnt pathway (Saxena et al., 2007; Sladek et al., 2007; Voight et al., 2010; Zeggini et al., 2008), most notably the Wnt effector TCF7L2 (da Silva Xavier et al., 2009; Grant et al., 2006).
Our data demonstrate that SFRP4 is present in both α and β cells and is released from islets, probably in a constitutive fashion. The gene coexpression module containing SFRP4 was enriched for IL-1-related genes, and we found that the expression and release of SFRP4 from islets was stimulated by IL-1β. Interestingly, analysis of β cell-enriched areas of pancreatic sections by laser-capture microdissection has shown that several inflammatory genes that are induced by IL-1β are overexpressed in T2D (Igoillo-Esteve et al., 2010). Moreover, IL-1β antagonism improves glycemia and β cell function (Cavelti-Weder et al., 2012; Dinarello et al., 2010; Larsen et al., 2007). The present data explain previous observations that IL-1β inhibits glucose-stimulated Ca²⁺-uptake and insulin release by a pertussis toxin-sensitive mechanism (Helqvist et al., 1989; Rabuazzo et al., 1995; Thaik et al., 1995). Since SFRP4 is associated with several inflammatory mediators in human islets it cannot be excluded that other cytokines in addition to IL-1β also affect SFRP4 release.

Secreted frizzled-related proteins (SFRPs) have a C-terminal netrin-like domain, which binds heparin and heparan sulfate proteoglycans in the extracellular matrix and facilitates the accumulation of SFRPs at high local concentrations at the site of secretion (Bafico et al., 1999; Salic et al., 1997; Uren et al., 2000). This makes it likely that the intraislet concentration of SFRP4 is considerably higher than that in serum, and our measurements of the amount of SFRP4 released from islets (~1 pg/islet over 24 hr; Figure S4F) clearly suggest that the concentration used in the experiments (30 nM) is in the physiological range.

SFRP4 in serum was associated with elevated fasting glucose and reduced disposition index. However, it was also associated with impaired insulin sensitivity, indicating that the protein could...
have a plethora of metabolic effects and might be released from several tissues involved in glucose homeostasis. It is interesting to note from the siRNA experiments targeting SFRP4 that relatively modest changes in the levels of released SFRP4 (~30% reduction using siRNA in mouse islets) can cause pronounced alterations of islet function. The elevation of SFRP4 in serum several years before the diagnosis highlights the protein as a potential biomarker for the low-grade islet inflammation that is commonly seen in T2D, and it might prove useful for early disease detection in combination with glucose and other biomarkers. Finally, our data raise the exciting possibility that SFRP4 could be a therapeutic target for specific treatment of islet dysfunction.

**EXPERIMENTAL PROCEDURES**

### Human Islets

Experimental procedures were approved by the local ethical committee. Donated human pancreatic islets were obtained (with research consent) from the Nordic Network for Clinical Islet Transplantations (Professor O. Korsgren). Donors with known T2D or HbA1c > 6.0% and no GAD antibodies were defined as having T2D. There was no difference in islet purity between ND and T2D donors. See Tables S1 and S5 for data on donors.

### Insulin Secretion and Exocytosis Measurements

Insulin secretion from isolated islets and β cell exocytosis were measured as previously described (Rosengren et al., 2010).

### Pancreatic Islet Cell Viability

Islet cell viability was measured using an Aqueous One Solution Cell Proliferation Assay Reagent (Promega, Sweden) according to the manufacturer’s instructions.

### RNA Interference

Islets or cell lines were transfected with siRNAs using Lipofectamine RNAiMax (QIAGEN). Gene expression was measured by qPCR using TaqMan (Applied Biosystems).

### TCF/LEF Activity

TCF/LEF activity in INS832/13 cells was measured with the Wnt Cignal TCF/LEF Activity Assay Kit (SABiosciences) according to the manufacturer’s instructions.

### [Ca++], Measurements

[Ca++] was estimated by Fura-2 and dual-wavelength fluorimetry.

### SFRP4 Content

SFRP4 in serum and islet incubation medium was measured with a human SFRP4 ELISA kit (Nordic Diagnostica, Sweden) according to the manufacturer’s instructions.

### Quantitative PCR

RNA was extracted with chloroform precipitation using the mRNeasy Kit (QIAGEN). Gene expression was measured by qPCR using TaqMan (Applied Biosystems).

### In Vivo Experiments

Female NMRI mice (30 g) were injected intraperitoneally with SFRP4 (200 μg/kg in PBS) or PBS at 24, 16, and 8 hr before intravenous glucose tolerance tests (IVGTT). IVGTT was performed after 4 hr fasting.

### Individuals from the Botnia Study

Sample rooms were obtained from individuals (88 in the initial analysis and 138 in the replication) participating in the Botnia Study (Groop et al., 1996). Individuals between 18 and 70 years were invited to prospective visits.

### Statistical Analyses

Student’s t test was used for comparisons of data from the cellular and animal experiments. For experiments using islets or β cells from human donors, the average for each donor under the different conditions was used in the analyses and compared with paired Student’s t test. One-sided tests were used for the replication analyses, and two-sided tests were used otherwise.

The SFRP4 concentration in serum showed non-Gaussian distribution. Gaussian distribution was obtained using logarithm transformation. All statistical analyses of serum SFRP4 were therefore performed using In transformed data. All statistical analyses were performed using IBM SPSS Statistics (ver 20.0).

### ACCESSION NUMBERS

All human islet microarray data are MIAME compliant, and the raw data have been deposited in a MIAME database (GEO, accession number: GSE41762).

### SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, 13 tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2012.10.009.

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### REFERENCES


