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Bernadette Neve, Martin E Fernandez-Zapico, Vered Ashkenazi-Katalan, Christian Dina, Yasmin H Hamid, et al.. Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102 (13), pp.4807-12. 10.1073/pnas.0409177102 . hal-00174488

**HAL Id: hal-00174488**

**<https://hal.science/hal-00174488>**

Submitted on 1 Jun 2020

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# Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function

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Edited by Donald F. Steiner, University of Chicago, Chicago, IL, and approved February 7, 2005 (received for review December 9, 2004)

**KLF11 (TIEG2) is a pancreas-enriched transcription factor that has elicited significant attention because of its role as negative regulator of exocrine cell growth *in vitro* and *in vivo*. However, its functional role in the endocrine pancreas remains to be established. Here, we report, for the first time, to our knowledge, the characterization of KLF11 as a glucose-inducible regulator of the insulin gene. A combination of random oligonucleotide binding, EMSA, luciferase reporter, and chromatin immunoprecipitation assays shows that KLF11 binds to the insulin promoter and regulates its activity in beta cells. Genetic analysis of the *KLF11* gene revealed two rare variants (Ala347Ser and Thr220Met) that segregate with diabetes in families with early-onset type 2 diabetes, and significantly impair its transcriptional activity. In addition, analysis of 1,696 type 2 diabetes mellitus and 1,776 normoglycemic subjects show a frequent polymorphic Gln62Arg variant that significantly associates with type 2 diabetes mellitus in North European populations (OR = 1.29, *P* = 0.00033). Moreover, this variant alters the corepressor mSin3A-binding activity of KLF11, impairs the activation of the insulin promoter and shows lower levels of insulin expression in pancreatic beta cells. In addition, subjects carrying the Gln62Arg allele show decreased plasma insulin after an oral glucose challenge. Interestingly, all three nonsynonymous KLF11 variants show increased repression of the catalase 1 promoter, suggesting a role in free radical clearance that may render beta cells more sensitive to oxidative stress. Thus, both functional and genetic analyses reveal that KLF11 plays a role in the regulation of pancreatic beta cell physiology, and its variants may contribute to the development of diabetes.**

insulin | polymorphisms | TGF- $\beta$  | type 2 diabetes

Components of both the exocrine and endocrine pancreas are affected by diseases, e.g., pancreatic cancer and type 2 diabetes mellitus (T2DM), which severely compromise both the quality and span of human life. Both glandular compartments share the same cellular origin and early morphogenetic pathways, suggesting a close functional and pathophysiological relationship. For instance, the exocrine-specific transcription factor p48 and the endocrine-specific pancreatic duodenal homeobox gene 1 (PDX-1) are both expressed in the common cell precursor (1); and, under pathological conditions their compartmentalization may be lost, as exemplified by the

detection of PDX-1 in pancreatic cancer (2). In fact, T2DM is both a common feature and a risk factor for the subsequent development of pancreatic cancer (3, 4). The TGF- $\beta$ -inducible transcription factor KLF11 regulates exocrine cell growth and behaves as a tumor suppressor in pancreatic cancer (ref. 5 and M.E.F.-Z. and R.U., unpublished observation). Because the TGF- $\beta$  signaling pathway is also a major regulator of endocrine cell fate (1, 6), the current study has been designed to define the role of the Sp1-like transcription factor KLF11 in the biology of the endocrine beta cell. Interestingly, several beta cell-specific genes display a potential binding site for Sp1 proteins in their promoter, suggesting that KLF11 is a potential endocrine regulator. Because the TGF- $\beta$  signaling pathway is crucial for pancreatic development and the impaired beta cell function observed in maturity-onset diabetes of the young (MODY) is caused by mutations in transcription factor genes that are also important in pancreas development [hepatocyte nuclear factors and PDX-1 (7), *KLF11* may also be a candidate gene for predisposition to diabetes. Indeed, here we show that KLF11 is a glucose-induced regulator of the insulin gene and functional *KLF11* gene variants are significantly associated with diabetes. These data, in combination with previous studies on KLF11, outline a molecular pathway important in both pancreatic cancer and diabetes.

## Methods

**Promoter Sequence Analysis.** For EMSAs, purified GST-fusion proteins of KLF11 (8) were incubated in a buffer containing 4% glycerol, 1 mM MgCl<sub>2</sub>, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 50  $\mu$ g/ml poly dI-dC (Promega) for 10 min at room temperature, and incubated for an additional 20 min with 0.035 pmol of end-labeled probes. The reactions were analyzed

This paper was submitted directly (Track II) to the PNAS office.

Freely available online through the PNAS open access option.

Abbreviations: ChIP, chromatin immunoprecipitation; T2DM, type 2 diabetes mellitus; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; SOD, superoxide dismutase; LD, linkage disequilibrium; BMI, body mass index; PDX-1, pancreatic duodenal homeobox gene 1.

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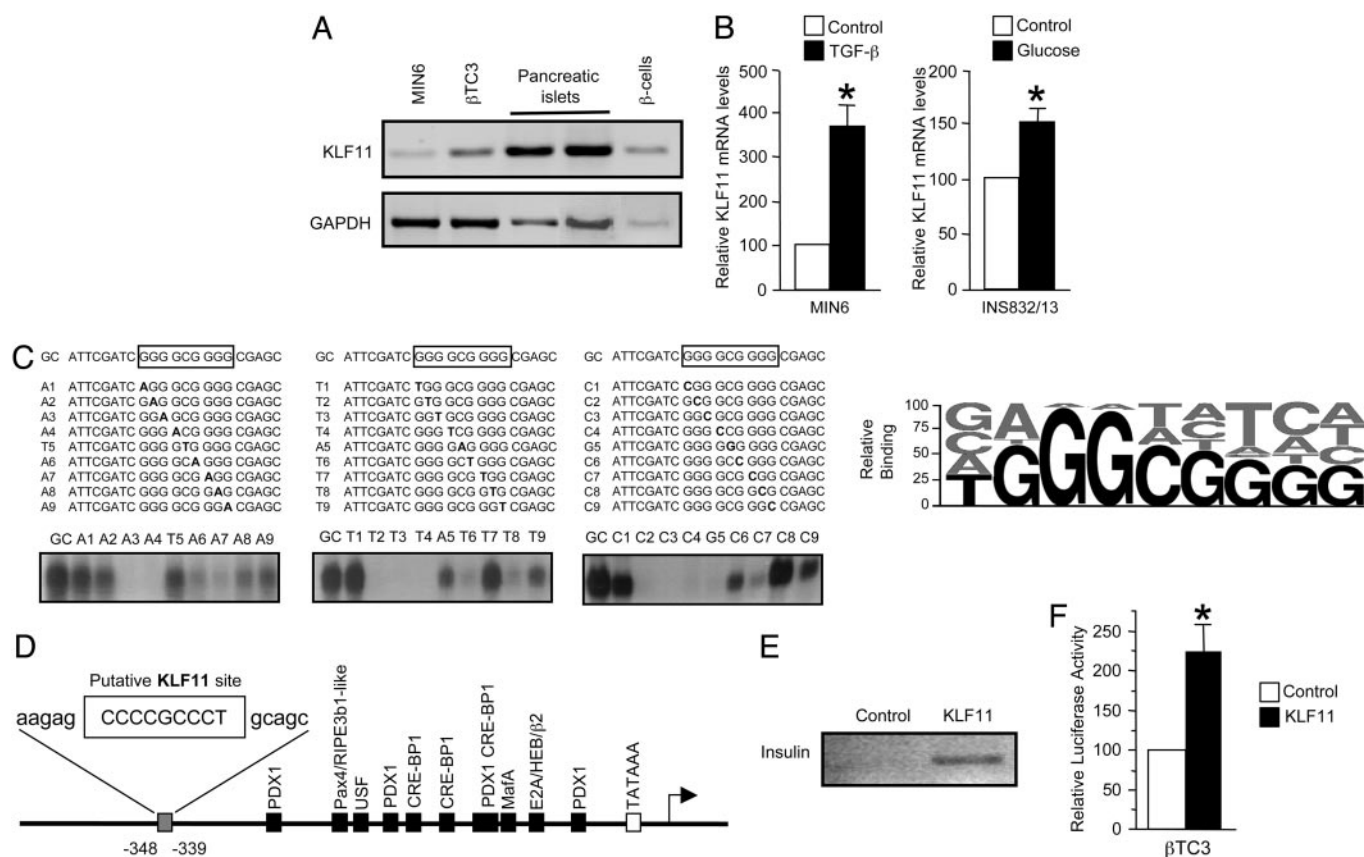
by electrophoresis on 4% nondenaturing polyacrylamide gel followed by autoradiography. Site-directed mutagenesis of the GC box was generated by replacing each base with A, T, or C and densitometric units of each protein/probe complex were determined by using NIH IMAGE 1.6.1 software. The relative binding specificity for an individual nucleotide at each position was calculated by using the following equation: (dNTP units)<sub>n</sub>/(SdNTPs units)<sub>n</sub>, where *n* = nucleotide position within the 9 bp GC box and is graphically represented by the height of letters, using the program WEBLOGO (9).

**Chromatin Immunoprecipitation (ChIP).** At 24 h after transfection with KLF11 FLAG-tagged plasmids (8),  $\beta$ TC3 cells were cross-linked with formaldehyde for 20 min at 25°C, harvested in SDS-lysis buffer (Upstate Biotechnology, Lake Placid, NY), and sheared to fragment DNA to  $\approx$ 500 bp. Samples were then immunoprecipitated by using an agarose-conjugated anti-FLAG antibody (Sigma-Aldrich, St. Louis) or agarose beads alone at 4°C overnight, washed, and eluted by using the ChIP kit (Upstate Biotechnology). Crosslinks were removed at 65°C for 4 h, and immunoprecipitated DNA was purified by using phenol/chloroform extraction and ethanol precipitation. Then, a 200-bp region of the insulin promoter was amplified by PCR, using the following primers 5'-GGC-CATCTGCCTACCCACCC and 5'-AGGCCCAAAGAG-GAGAGTACATAC and visualized by 2.5% agarose gel.

**Reporter Assays.** The KLF11 variants were generated following the recommendations of the Stratagene QuikChange kit. Then,  $\beta$ TC3

cells were transfected by using TransFast transfection (Promega, Charbonnières, France). The KLF11 constructs were cotransfected with the Gal4 reporter construct (10), a luciferase reporter vector containing six tandem repeats of KLF11-binding sites (10), the human insulin promoter, or catalase 1 promoter (5), and the luciferase activity was measured as described (5). Association of endogenous mSin3A with KLF11 was analyzed by immunoprecipitation assays (ref. 8 and *Supporting Methods*, which is published as supporting information on the PNAS web site).

**Genetic Analysis and Study Population.** The identification and genotyping of genetic variants and calculation of logarithm of odds scores are described in *Supporting Methods* and Table 4, which is published as supporting information on the PNAS web site. The 96 normoglycemic control and 66 T2DM subjects of the pilot study were included in the familial study with 313 French cases from T2DM diabetes multiplex families that had at least one affected first-degree relative, and 313 control subjects (spouses) that were selected to match the cases for T2DM risk factors (Table 5, which is published as supporting information on the PNAS web site). The second study consisted of 1,383 consecutive T2DM patients and 1,463 control subjects older than 45 years of age from general populations (Table 5). Association was assessed by  $\chi^2$  analysis (11). Logistic regression and ANOVA were performed by using SPSS software (SPSS, Chicago). Tests for deviation from Hardy-Weinberg equilibrium (12) and for association were adapted from Sasieni (13).



**Fig. 1.** Role of KLF11 in pancreatic beta cells. (A) KLF11 mRNA expression in the beta cell lines Min6 and  $\beta$ TC3 in two independent isolations of human pancreatic islets, and in human beta cells isolated by zinc selection (14). (B) Quantitative PCR measurement of KLF11 mRNA expression in both Min6 and INS832/13 cells. (C) EMSA with the KLF11-GST fusion protein showing binding to the wild-type and mutated GC boxes. (Right) A representation of the relative percentage of DNA binding (Lower). (D) Position of the putative KLF11-binding sequence of the insulin promoter. (E) Binding of KLF11 to insulin promoter analyzed by ChIP assays in  $\beta$ TC3 cells. (F) Luciferase assays of Flag-tagged KLF11 transiently cotransfected with the human insulin promoter reporter construct in  $\beta$ TC3 cells cultured in high-glucose medium (*n* = 6). \*, significantly different (*P* < 0.05).









