Brief Genetics Report

Transcription Factor *TCF7L2* Genetic Study in the French Population

Expression in Human β-Cells and Adipose Tissue and Strong Association With Type 2 Diabetes

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Recently, the transcription factor 7-like 2 (*TCF7L2*) gene has been associated with type 2 diabetes in subjects of European origin in the DeCode study. We genotyped the two most associated variants (rs7903146 and rs12255372) in 2,367 French type 2 diabetic subjects and in 2,499 control subjects. Both the T-allele of rs7903146 and the T-allele of rs12255372 significantly increase type 2 diabetes risk with an allelic odds ratio (OR) of 1.69 (95% CI 1.55–1.83) ($P = 6.0 \times 10^{-35}$) and 1.60 (1.47–1.74) ($P = 7.6 \times 10^{-28}$), respectively. In nonobese type 2 diabetic subjects (BMI <30 kg/m^2, $n = 1,346$), the ORs increased to 1.89 (1.72–2.09) ($P = 2.1 \times 10^{-38}$) and 1.79 (1.62–1.97) ($P = 5.7 \times 10^{-31}$), respectively. The rs7903146 T at-risk allele associates with decreased BMI and earlier age at diagnosis in the type 2 diabetic subjects ($P = 8.0 \times 10^{-3}$ and $P = 3.8 \times 10^{-4}$, respectively), which is supported by quantitative family-based association tests. *TCF7L2* is expressed in most human tissues, including mature pancreatic β-cells, with the exception of the skeletal muscle. In the subcutaneous and omental fat from obese type 2 diabetic subjects, *TCF7L2* expression significantly decreased compared with obese normoglycemic individuals. During rat fetal β-cell differentiation, *TCF7L2* expression pattern mimics the key marker *NGN3* (neurogenin 3), suggesting a role in islet development. These data provide evidence that *TCF7L2* is a major determinant of type 2 diabetes risk in European populations and suggests that this transcription factor plays a key role in glucose homeostasis. *Diabetes* 55: 2903–2908, 2006

To date, positional cloning for type 2 diabetes genes has not been very successful in detecting type 2 diabetes putative susceptibility genes. Although several positive reports have emerged (1,2), few have been consistently replicated (3). Several studies have shown linkage between type 2 diabetes and chromosome 10q in Mexican-American, French, English, and Icelandic populations (4–7) with a strong association between type 2 diabetes and variation in the transcription factor 7-like 2 (*TCF7L2*) gene found in Icelandic, Danish, and American populations (8). Little is known about the physiological implication of this transcription factor in glucose homeostasis. It has been suggested that intestinal proglucagon gene expression may be regulated by the Wnt/*TCF7L2* pathway in enteroendocrine cells (9). Thus, *TCF7L2* variants may modify type 2 diabetes susceptibility through modulation of glucagon-like peptide-1 (GLP-1) secretion. Because French type 2 diabetic families exhibit evidence for linkage in a chromosome 10q region encompassing the *TCF7L2* locus, we assessed the contribution of *TCF7L2* genetic variation to type 2 diabetes genetic risk. Tissue profiling analyses were also performed in rodents and humans to help understand the regulation of *TCF7L2* and its involvement in the physiology of type 2 diabetes.

Among the previously reported *TCF7L2* variants, the association between the T-allele of rs7903146 and the T-allele of rs12255372 have been replicated (8). These two variants were genotyped in 2,367 French type 2 diabetic subjects and in 2,499 normoglycemic nonobese control middle-aged individuals (fasting glucose <5.6 mmol/l and BMI <30 kg/m^2) (Table 1). We found a strong association between type 2 diabetes and the two variants in our case-control study (Table 2). The genotypic distributions for both variants were in Hardy-Weinberg equilibrium, and the T-alleles of rs7903146 and rs12255372 were both found to significantly increase type 2 diabetes risk with an allelic odds ratio (OR) of 1.69 (95% CI 1.55–1.83) ($P = 6.0 \times 10^{-35}$) and 1.60 (1.47–1.74) ($P = 7.6 \times 10^{-28}$), respectively. Testing for the two-variant haplotype did not produce a better result than the two variants analyzed individually.

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FBAT, family-based association test; GLP-1, glucagon-like peptide 1; PAR, population-attributable risk; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

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TABLE 1
Clinical characteristics of the type 2 diabetes case-control groups

	Control	All type 2 diabetes	Nonobese type 2 diabetes
n	2,499	2,367	1,315
Sex ratio (male:female)	1,020:1,479	1,402:965	856:459
Age at examination (years)	57.09 ± 9.98	58.61 ± 11.12	59.78 ± 11.02
Age at diagnosis (years)	—	47.79 ± 10.13	47.98 ± 10.30
BMI (kg/m^2)	22.76 ± 2.57	30.01 ± 5.93	26.08 ± 3.43

Data are means ± SD. Nonobese type 2 diabetes is a subgroup of all type 2 diabetes (BMI <30 kg/m^2).

Analyzing the 1,346 nonobese type 2 diabetic subjects (BMI <30 kg/m^2) by themselves increases the allelic OR to 1.89 (1.72–2.09) ($P = 2.1 \times 10^{-38}$) and 1.79 (1.62–1.97) ($P = 5.7 \times 10^{-31}$), respectively.

As the two variants exhibit strong linkage disequilibrium ($r^2 = 0.77$, $D' = 0.89$), further analyses focused on rs7903146 (the single nucleotide polymorphism [SNP] with the strongest association to type 2 diabetes). The population-attributable risk (PAR) for rs7903146 was estimated at 31% using our case-control sample. When the allelic OR is estimated in the nonobese subjects versus the control subjects (BMI <30 kg/m^2), the attributable risk reaches 37.7%. The T-allele frequency was 30.5% in control individuals and 42.5% in type 2 diabetic subjects. We tested departure from the additive model in a logistic regression framework, where an index variable identifies heterozygous individuals. No significant deviation was observed ($P = 0.74$), supporting the hypothesis of a risk increase proportional to the number of variant alleles (genotypic OR = 1.66 for C/C vs. C/T and genotypic OR = 2.86 for C/C vs. T/T). In this study, there is a discrepancy in sex between type 2 diabetic and control subjects, but no statistical heterogeneity was found between male and female ORs ($P = 0.32$). The adjusted allelic OR, using the Mantel-Haenszel approach, was 1.70 (1.56–1.85) ($P = 3.2 \times 10^{-35}$), very close to the allelic OR observed with the pooled data. There is evidence of an association of the at-risk allele with lower BMI in the type 2 diabetic group (30.40 ± 6.30 kg/m^2 for C/C, 30.01 ± 5.71 kg/m^2 for C/T, and 29.29 ± 5.75 kg/m^2 for T/T; $P = 8.0 \times 10^{-3}$), and T-allele carriers were 2.4 years younger at type 2 diabetes diagnosis (48.78 ± 9.79 years of age for C/C, 47.62 ± 10.29 years of age for C/T, and 46.42 ± 10.14 years of age for T/T; $P = 3.8 \times 10^{-4}$). There is no evidence of an association with the variant and type 2 diabetes complications such as history of coronary heart disease or severe retinopathy or nephropathy (data not shown). In the individuals without diabetes, there was no evidence of an association between the T at-risk allele and homeostasis model assessment–derived β-cell function (fasting insulinemia [μU/ml] × 20)/(fasting glycemia [mmol/l] − 3.5), homeostasis model assessment for insulin resistance {[fasting insulinemia (μU/ml) × fasting glycemia (mmol/l)]/22.5}, fasting insulinemia, fasting glycemia, total cholesterol, LDL cholesterol, HDL cholesterol, glycated hemoglobin, BMI, or triglycerides at baseline and during the 9-year follow-up (online appendix Table 1 [available at http://diabetes.diabetesjournals.org]). There is a trend for a decrease in fasting insulinemia between CC and TT individuals (39.17 ± 19.01 pmol/l for C/C, 38.08 ± 25.79 pmol/l for C/T, and 35.56 ± 19.64 pmol/l for T/T; $P = 0.08$).

We investigated the familial association with type 2 diabetes–associated traits in the 148 type 2 diabetes pedigrees, which showed evidence of linkage on chromosome 10q in 633 individuals, 432 with type 2 diabetes. We observed an overtransmission of the T at-risk allele in affected offspring (76.9% in 60 informative pedigrees, $P = 1.8 \times 10^{-4}$). The association with BMI and the age at type 2 diabetes diagnosis was confirmed by family-based association tests (FBATs) ($P = 0.01$ and $P = 9.9 \times 10^{-4}$, respectively). We determined that the *TCF7L2* gene variant does not explain the linkage signal by testing the correlation between familial at-risk allele frequency and familial linkage score (nonparametric linkage) at different locations. This correlation was first tested at the marker position where the maximal logarithm of odds score is found (5) (D10S1655) and then at the marker closest to the

TABLE 2
Genotypic distributions and ORs of rs7903146 and rs12255372 among control and type 2 diabetes

	Frequency				
SNP/genotype	Control (*n*)	All type 2 diabetes (*n*)	Nonobese type 2 diabetes (*n*)	Allelic OR (95% CI)	*P* value
rs7903146					
C/C	1,208	787	396	1.69 (1.55–1.83)*	6.0×10^{-35}
C/T	1,060	1,149	644		
T/T	231	431	275	1.89 (1.72–2.09)†	2.1×10^{-38}
MAF (%)	30.5	42.5	45.4		
rs12255372					
G/G	1,243	858	443	1.60 (1.47–1.74)*	7.6×10^{-28}
G/T	1,031	1,131	632		
T/T	209	373	242	1.79 (1.62–1.97)†	5.7×10^{-31}
MAF (%)	29.2	39.7	42.4		

Nonobese type 2 diabetes is a subgroup of all type 2 diabetes (BMI <30 kg/m^2). r^2 and D′, i.e., linkage disequilibrium values between both the T-alleles of rs7903146 and rs12255372, = 0.77 and 0.89, respectively. The genotypic distributions for both variants are in Hardy-Weinberg equilibrium. *Control vs. all type 2 diabetes; †control vs. nonobese type 2 diabetes. MAF, minor allele frequency.

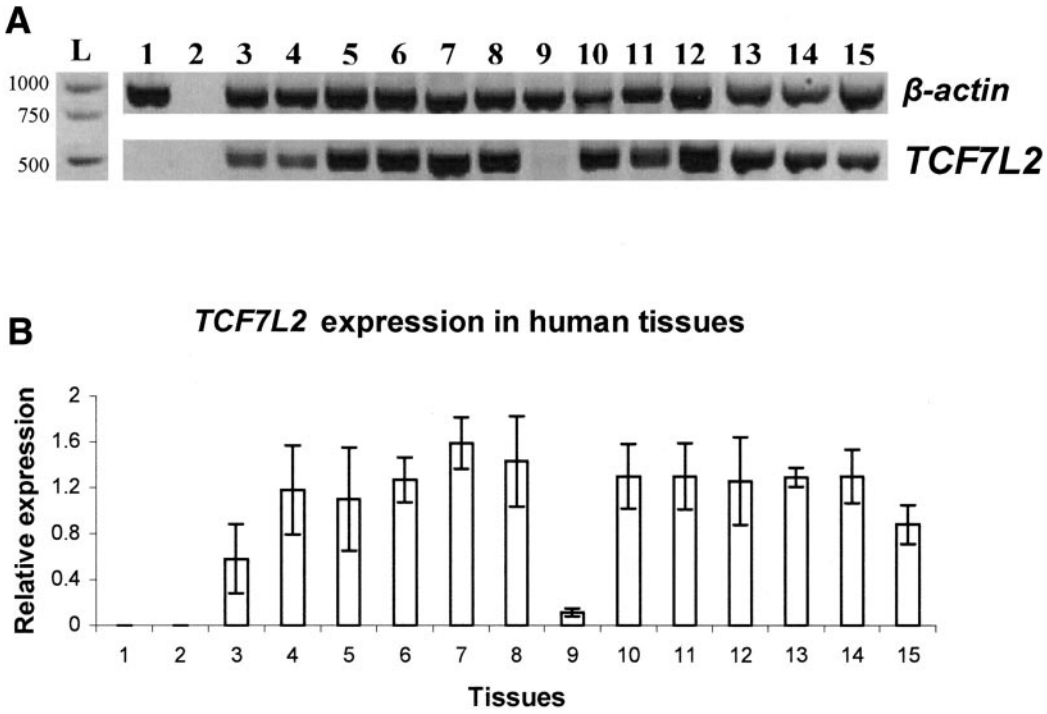

FIG. 1. *TCF7L2* expression in the human multiple tissue cDNA panel, where 1 = positive control for β-actin, 2 = negative control, 3 = cDNA control, 4 = heart tissue, 5 = placenta, 6 = lung, 7 = brain, 8 = liver, 9 = skeletal muscle, 10 = kidney, 11 = pancreas, 12 = adipocyte, 13 = omental adipose tissue, 14 = subcutaneous adipose tissue, and 15 = β-cells. *TCF7L2* expression was measured in the human multiple tissue cDNA panel in triplicate (n = 3). β-Actin was used as internal control. *A*: MTC panel PCR bands. *B*: Adjusted expression mean values.

TCF7L2 gene (D10S597). Lack of evidence of a correlation showed that the SNP does not explain the linkage.

Figure 1 shows that *TCF7L2* is highly expressed in most human tissues, including mature pancreatic β-cells, with the exception of the skeletal muscle. In human subcutaneous and omental adipose tissues, *TCF7L2* expression is significantly impaired in obese subjects developing type 2 diabetes (P = 0.03 for omental fat and P = 0.02 for subcutaneous fat) (Fig. 2*A*). During rat fetal β-cell differentiation, *TCF7L2* expression pattern mimics neurogenin 3 (*NGN3*) (early-step key marker) ($P < 0.02$) but not paired box gene 6 (*Pax6*) (late-step key marker), suggesting a role in the gene expression cascade previously described in islet development (10) (Fig. 2*A* and *B*). Pancreatic *TCF7L2* expression does not vary in *NGN3* knock-out mice, possibly because it is expressed in other tissues, as well as the endocrine pancreas (data not shown).

DISCUSSION

Our data provides strong evidence that the transcription factor *TCF7L2* is a major susceptibility gene for type 2 diabetes in the French population. This is supported by both case-control analysis and familial transmission disequilibrium test (TDT) performed on 4,866 subjects, as well as FBAT analyses that exclude obvious stratification biases. However, the FBAT analysis was not completely independent from the case-control study, since 126 unrelated type 2 diabetic subjects from the case-control study were from the 148 pedigrees. In addition to a significant association for the rs7903146 T at-risk allele (allelic OR = 1.7, $P = 6.0 \times 10^{-35}$), 31% of diabetes cases can be attributable to the presence of the T at-risk allele of the rs7903146 variant, which suggests that *TCF7L2* is one of the strongest type 2 diabetes polygenes found to date. The contribution of *TCF7L2* is even higher in nonobese (PAR = 37.7%). However, to calculate the PAR, the population genotypic frequencies were estimated by the control genotypic frequencies, and the PAR was probably overestimated due to clinically selected type 2 diabetic subjects. A more appropriate PAR estimation requires a larger general population analysis, which is currently underway. We found that the rs7903146 T at-risk allele modulates BMI in type 2 diabetic subjects (-1.1 kg/m^2) and decreases the age at diagnosis by 2.4 years (further supported by quantitative FBAT). The strong association of rs7903146 T at-risk allele with type 2 diabetes in nonobese subjects makes it unlikely that the *TCF7L2* diabetogenic effect directly involves insulin sensitivity or fat deposition.

The lack of association with the linkage peak suggests that other variants in the same region are also associated with type 2 diabetes, but as the linkage peak is weak (5), further studies are required in other pedigrees in regions of strong linkage in chromosome 10q (4,6,7). There was no evidence of an association with any metabolic parameter, but there was a trend for a decrease in fasting insulinemia in T at-risk allele carriers within the 2,499 control subjects (online appendix Table 1). This does not provide evidence of the gene effect on insulin secretion/sensitivity; therefore, further investigation in a sample where oral glucose tolerance is measured is required.

Recently, Yi et al. (9) found that *TCF7L2* was not expressed in murine pancreatic islets but suggested that it may activate the proglucagon gene in enteroendocrine cells. In contrast, we report significant expression in human fluorescence-activated cell sorter–purified pancreatic β-cells, suggesting that this transcription factor may be involved in β-cell development and/or function. A putative role in β-cell differentiation is suggested by the strong correlation between *NGN3* and *TCF7L2* expression

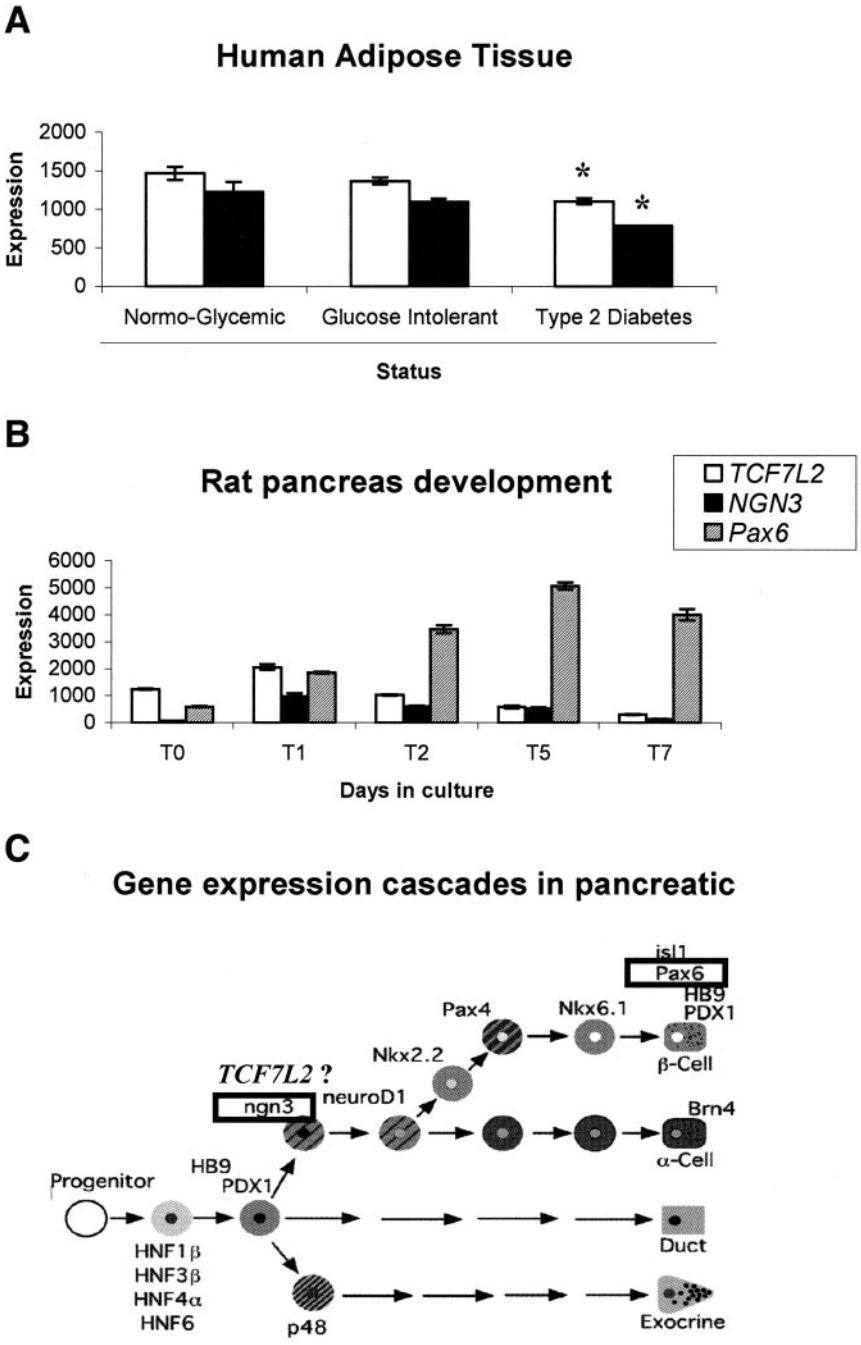

FIG. 2. ***TCF7L2*** **gene expression in human adipose and rat pancreatic tissues. Each condition was done in triplicate (*n* = 3) in Affymetrix microarrays (HG-U133_Plus2 and RAE230, respectively). *A*: Human subcutaneous (□) and omental (■)** ***TCF7L2*** **expression significantly decreases between normoglycemia and type 2 diabetes (*P* = 0.03 for omental and *P* = 0.02 for subcutaneous) and between glucose intolerance type 2 diabetes (*P* = 0.02 for omental and *P* = 0.04 for subcutaneous). No difference was found between normoglycemia and glucose intolerance in the two adipose tissues. *B*: Rat embryo epithelium** ***TCF7L2*** **expression significantly decreased with the differentiation in β-cells (*P* < 0.02) like** ***NGN3*****. *C*:** ***NGN3*** **is expressed at the beginning of β-cell differentiation, whereas** ***Pax6*** **is expressed at the last step of the β-cell differentiation according to Wilson et al. (10). HNF, hepatocyte nuclear factor; PDX, pancreatic and duodenal homeobox factor. *P < 0.05.**

during the early steps of rat pancreas development. In this regard, attenuated Wnt signaling might restrict pancreatic growth (11).

We found that the *TCF7L2* gene is significantly expressed in human tissues that are vital for glucose homeostasis, including visceral and subcutaneous fat. Its expression is downregulated in obese subjects developing type 2 diabetes, but its relevance in physiology still requires evaluation. *TCF7L2* expression needs to be measured in other tissues to assess the tissue specificity of such differential expression. Recent work suggests that the Calpain system is involved in the constitutive regulation of β-catenin signaling functions (12), which raises the hypothesis of a potential interaction between *TCF7L2* and *Calpain-10*.

In conclusion, our results not only replicate the Icelandic data but also provide strong evidence that *TCF7L2* is a major determinant of diabetes risk in the European population. As previously suggested, an additive model of inheritance fits well with our data (8), which triples the

risk of developing type 2 diabetes for homozygous T/T carriers (8.9% of type 2 diabetic subjects). Further work remains to be completed to evaluate the contribution of *TCF7L2* in other ethnic groups and to elucidate the pathways controlled by this transcription factor. Its β-cell expression and its downregulation with type 2 diabetes occurrence suggest that it plays an important role in glucose homeostasis.

RESEARCH DESIGN AND METHODS

The clinical characteristics of all French type 2 diabetic subjects are described in Table 1. Type 2 diabetic subjects were diagnosed with diabetes according to World Health Organization criteria or were currently being treated for diabetes. Altogether, 355 probands from families with type 2 diabetes were recruited by the Centre National de la Recherche Scientifique–Institut Pasteur Unit in Lille, and 2,012 type 2 diabetic subjects were patients of the Endocrinology-Diabetology Department at Corbeil-Essonnes Hospital. Two groups of healthy control subjects of French origin were analyzed (fasting glucose <5.6 mmol/l, age at exam >40 years, BMI <30 kg/m^2), including 228 normoglycemic subjects from the family collection recruited in Lille and 2,271 normoglycemic nonobese control subjects from the DESIR (Angers) prospective population-based cohort (13) were used for replication. The nonobese subgroup of the type 2 diabetic subjects (n = 1,346, BMI <30 kg/m^2) was analyzed to estimate the association of the variants with the nonobese type 2 diabetic subjects. Genotypic homogeneity was tested by χ^2 test, and no significant difference was found between control subjects (Angers vs. Lille) or between type 2 diabetic subjects (Corbeil-Essones vs. Lille). In this study, 8.8% of type 2 diabetic subjects have a Maghrebian origin. Analyses have been repeated with Maghrebian or European subjects only (online appendix Tables 2 and 3). Genotypic homogeneity was tested by χ^2 test, and no significant difference was found between type 2 diabetic subjects (Maghrebian vs. European). In addition, familial association tests were performed in 148 French pedigrees recruited by UMR8090 that had been previously scanned for linkage with type 2 diabetes (5). These families consisted of a total of 633 individuals, of whom 432 presented with diabetes (sex ratio male:female 198:234, mean age at diagnosis 49.5 ± 10.6 years, and mean BMI 27.9 ± 4.5 kg/m^2), 72 were glucose intolerant (sex ratio 36:36, mean age at diagnosis 59.1 ± 9.6 years, and mean BMI 27.4 ± 4.7 kg/m^2), and 129 were normoglycemic subjects (sex ratio 40:89 and mean BMI 25.1 ± 4.1 kg/m^2). A total of 126 unrelated type 2 diabetic individuals from the case-control study were from the 148 pedigrees with type 2 diabetes. The genetic study was approved by the ethical committee of Hotel-Dieu in Corbeil-Essonnes and CHRU in Lille, and informed consent was obtained from all participants.

Genotyping methods. High-throughput genotyping of SNPs rs12255372 and rs7903146 was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The PCR primers and TaqMan probes were designed by Primer Express and optimized according to the manufacturer's protocol. There was a 98% genotyping success rate, and the genotyping error rate was assessed by sequencing 384 control and 384 type 2 diabetic individuals and by regenotyping a random 10% of subjects. No difference was found with the first genotyping results; therefore, the genotyping error rate was 0%.

***TCF7L2* mRNA expression.** Human cDNAs from the human multiple tissue cDNA panel (BD Biosciences Clontech), fluorescence-activated cell sorter–purified pancreatic β-cells (provided by the Human Pancreatic Cell Core Facility, University Hospital, Lille, France), and subcutaneous and omental adipocytes were used for mRNA expression analysis (Fig. 1). β-Cell purity was confirmed by immunochemistry (98% insulin-positive cells) and PCR (absence of amplification with chymotrypsin primers, specific for exocrine cells, and presence of amplification with Pdx1 primers, specific for β-cells). The forward primer used for the multiple tissue cDNA panel was 5′-ACCATGTCCACCCC CTCACGCCTCT-3′, and the reverse was 5′-TGGGGCTTCTTCTTTTCTTCTTC CTTTTTG-3′. These two primers were high-performance liquid chromatography purified. PCR was performed in a 25-μl mixture containing 10 mmol/l Tris-HCl, 1.5 mmol/l MgCl2, 10 mmol/l of each dNTPs, 2.5 units *Taq* polymerase (Promega), 30 mmol/l of both forward and reverse primer, and 3 μl single-strand cDNA, using the hot-start PCR method of 95°C for 2 min, 45 cycles of 95°C for 30 s, 68°C for 2 min, 72°C for 2 min, and then 72°C for 10 min. PCR products were separated on 2% (wt/vol) agarose gel and visualized using ethidium bromide and ultraviolet transillumination, and β-actin amplification was used as internal control. Omental and subcutaneous adipose tissue from normoglycemic (n = 3, all women, aged 44 ± 7 years, BMI 39.2 ± 3.8 kg/m^2), glucose intolerant (n = 3, all women, aged 45 ± 7 years, BMI 45.3 ± 7.0 kg/m^2), and type 2 diabetic (n = 3, two women and one man, aged 55.7 ± 5.8 years, BMI 49.0 ± 7.4 kg/m^2) individuals were analyzed for *TCF7L2* expression in HG-U133_Plus2 (Affymetrix microarray) (Fig. 2*A*). Affymetrix chip HG-U133_Plus2 contains 15 probes for *TCF7L2* genes. For six probes, the signal is too low to detect any expression level. The probe "212761_at" was selected to represent *TCF7L2* gene, since it has the higher signal and was located in the 3′ untranslated region of the gene. Expressions were computed using the GeneChip Robust Multi-array Analysis method, and normalization was performed for each condition using the quantile method. Both methods are implemented using R language in the bioconductor package. *TCF7L2*, *Pax6*, and *NGN3* gene expressions were assessed in a rat pancreas development model in RAE230 (Affymetrix microarray). Rat pancreas embryonic samples were cultured in triplicate (n = 3 different embryos from the same mother) during different time lapses (0, 1, 3, 5, and 7 days), mimicking the endocrine cell differentiation and proliferation (Fig. 2*B*). Pancreatic *TCF7L2* expression was also measured in *NGN3* knockout mice by TaqMan (14).

Statistical methods. Tests for deviation from Hardy-Weinberg equilibrium and for association were performed with the De Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The PAR was based on a model-free hypothesis, with two level of exposition, heterozygous and homozygous variants. To calculate the PAR, the population genotypic frequencies were estimated by the control genotypic frequencies. A multivariate linear regression model taking into account age and sex was performed for BMI parameters. The same analysis was done with BMI adjustment for diabetes-onset parameters. Analysis on binary and quantitative traits was done by the TDT and quantitative TDT methods implemented in FBAT software. Evidence for association between identity-by-descent sharing and possession of the T at-risk allele was also investigated using the Genotype-IBD Sharing Test, which assigns family-specific weights based on the genotype of the affected family members and the model of interest (dominant, recessive, and additive) and tests for a positive correlation between this weight and the family-based identity-by-descent sharing as represented by the nonparametric linkage score. All reported P values are two tailed. SPSS 10.1 software was used for general statistical analyses.

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