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TCF7L2 gene expression in human visceral and subcutaneous adipose tissue is differentially regulated but not associated with type 2 diabetes mellitus

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Abstract

Variants in the *TCF7L2* gene have been associated with type 2 diabetes mellitus (T2DM), but the causal variant(s) is still unknown. We studied the *TCF7L2* messenger RNA (mRNA) expression in paired samples of visceral and subcutaneous adipose tissue from 49 subjects using quantitative real-time polymerase chain reaction and its relation to obesity and T2DM. All subjects were genotyped for the previously described *TCF7L2* diabetes risk variants. Independent of age, sex, obesity, and diabetes status, we found >3-fold higher *TCF7L2* mRNA expression in subcutaneous compared with visceral adipose tissue. There was no correlation between visceral and subcutaneous *TCF7L2* expression. No differences in adipose tissue *TCF7L2* mRNA expression levels were found between diabetic and nondiabetic subjects, or between lean and obese subjects (all *P*s > .05). In addition, there was no association between *TCF7L2* genetic variants and mRNA expression. Based on our data, *TCF7L2* mRNA expression is fat-depot specific but does not seem to provide the mechanistic link explaining genetic association with T2DM.

1. Introduction

The *TCF7L2* gene encodes a transcription factor that is involved in the regulation of cellular proliferation and differentiation [1]. Variants in the *TCF7L2* have been associated with an increased risk for type 2 diabetes mellitus (T2DM) [2,3]. These findings have been extended by showing that diabetes risk alleles predict the progression from impaired glucose tolerance (IGT) to diabetes prospectively in adults [4]. We could recently show that *TCF7L2* gene variants confer an increased risk for early impairment of glucose metabolism in obese children [5].

Recently, expression of *TCF7L2* in human β-cells and fat has been reported [6]. In obese subjects with T2DM, *TCF7L2* expression was significantly decreased in subcutaneous and omental fat compared with obese normoglycemic individuals [6]. However, these findings were based on expression studies performed on only 3 individuals per group and were not confirmed by quantitative polymerase chain reaction (PCR). We therefore studied *TCF7L2* messenger RNA (mRNA) expression in adipose tissue using quantitative real-time reverse transcriptase (RT) PCR in paired samples of visceral and subcutaneous adipose tissue from 49 subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance. Although association of *TCF7L2* variants with T2DM has been widely replicated, the causal variant(s) and its potential function are still unknown. Therefore, the second objective of our study was to investigate whether previously published *TCF7L2*

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diabetes risk alleles might explain variability in the *TCF7L2* mRNA expression.

2. Materials and methods

2.1. Subjects

Paired samples of visceral and subcutaneous adipose tissue were obtained from 49 white men (n = 23) and women (n = 26) who underwent open abdominal surgery for gastric banding, cholecystectomy, appendectomy, weight reduction surgery, abdominal injuries, or explorative laparotomy. The age ranged from 23 to 99 years, and body mass index (BMI) was from 22.7 to 49.6 kg/m^2. With oral glucose tolerance test (OGTT), we identified 16 individuals with either T2DM (n = 12) or IGT (n = 4). The OGTT was performed according to the criteria of the American Diabetes Association [7]. The test was carried out after an overnight fast with 75-g standardized glucose solution (Glucodex Solution 75g; Merieux, Montreal, Quebec, Canada). Venous blood samples were taken at 0, 60, and 120 minutes for measurements of plasma glucose concentrations. All subjects had a stable weight, with no fluctuations of >2% of the body weight for at least 3 months before surgery. Patients with malignant diseases or any acute or chronic inflammatory disease as determined by a leukocyte count >7000 gigaparticles per liter, C-reactive protein >5.0 mg/dL, or clinical signs of infection were excluded from the study. Samples of visceral and subcutaneous adipose tissue were immediately frozen in liquid nitrogen after explantation. The study was approved by the ethics committee of the University of Leipzig. All subjects gave written informed consent before taking part in the study.

2.2. Assays and measures of glucose metabolism and body fat content

Basal blood samples were taken after an overnight fast. Plasma insulin was measured with an enzyme immunometric assay for the IMMULITE automated analyzer (Diagnostic Products, Los Angeles, CA). The OGTT was performed after an overnight fast with 75-g standardized glucose solution (Glucodex Solution 75 g, Merieux) as previously described [8]. Insulin sensitivity was assessed with the euglycemic-hyperinsulinemic clamp method [9]. Percentage body fat was measured by dual-energy x-ray absorptiometry. In addition, abdominal visceral and subcutaneous fat area was calculated using computed tomographic scans at the level of L4 through L5.

2.3. Analysis of human TCF7L2 mRNA expression

Human *TCF7L2* mRNA expression was measured by using SYBR green methodology as previously described [10]. Briefly, gene expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler using the SYBR green methodology; and fluorescence was detected on an ABI PRISM 7500 Sequence Detector (Applied Biosystems, Darmstadt, Germany). Total RNA was isolated from paired subcutaneous and visceral adipose tissue samples using TRIzol (Life Technologies, Grand Island, NY), and 1 μg RNA was reverse transcribed with standard reagents (Life Technologies). From each RT-PCR, 2 μL was amplified in a 26-μL PCR using the Brilliant SYBR Green QPCR Core Reagent Kit from Stratagene (La Jolla, CA) according to the manufacturer's instructions. Samples were incubated in the ABI PRISM 7500 sequence detector for an initial denaturation at 95°C for 10 minutes followed by 40 PCR cycles, each cycle consisting of 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 1 minute. The following primers were used: human *TCF7L2* (accession no. NM_030756) 5' aaagcgcggccatcaac 3' (sense) and 5' cagctcgtagtatttcgcttgct 3' (antisense). Primer pairs were created using the Primer Express Software from Applied Biosystems. To make sure that primers only amplify complementary DNA, one primer lies across an intron/exon boundary. To avoid unspecific primer-dimer amplification, the secondary structure option was checked and amplification of specific transcripts was confirmed by melting curve profiles at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis. Human *TCF7L2* mRNA expression was calculated relative to the mRNA expression of 18S rRNA, determined by a premixed assay on demand for human 18S rRNA (PE Biosystems, Foster City, CA).

2.4. Genotyping of TCF7L2 variants

Genotyping of all 5 *TCF7L2* variants (rs7901695, rs7903146, rs12255372, rs11196205, and rs7895340) was performed using the TaqMan allelic discrimination assay (Applied Biosystems) on ABI PRISM 7500 sequence detector (Applied Biosystems). To assess genotyping reproducibility, a random ~10% selection of the samples was regenotyped in all single nucleotide polymorphisms (SNPs) with 100% concordance. Genotype data for all SNPs were consistent with Hardy-Weinberg equilibrium (all Ps > .05).

2.5. Statistical analyses

Data are shown as means ± SEM unless stated otherwise. Before statistical analysis, nonnormally distributed parameters (visceral and subcutaneous *TCF7L2* mRNA, fasting plasma insulin, 2-hour OGTT blood glucose, hemoglobin A_{1c}) were logarithmically transformed to approximate a normal distribution. Expression differences between visceral and subcutaneous adipose tissue were assessed using the paired Student t test. Associations of genetic variants with *TCF7L2* mRNA expression levels were assessed using generalized linear regression models under the additive and dominant/recessive mode of inheritance. All analyses were adjusted for age, sex, and BMI. Statistical analyses were

performed using the SPSS software package (version 11.5; SPSS, Chicago, IL) and statistical software from the SAS Institute (Cary, NC). P values < .05 were considered to be statistically significant.

3. Results

3.1. Visceral and subcutaneous TCF7L2 mRNA levels

Anthropometric and metabolic characteristics of subjects are summarized in Table 1. Analysis of paired samples of visceral and subcutaneous adipose tissue revealed >3-fold higher *TCF7L2* mRNA expression in subcutaneous compared with visceral adipose tissue in the entire study cohort (Fig. 1A) independently of sex (data not shown). Visceral *TCF7L2* mRNA expression did not correlate with age (R^2 = 0.037, P = .09) and subcutaneous expression (R^2 = 0.021, P = .32). To investigate whether the expression differences are explained by variability in body fat mass or fat distribution, we performed additional analyses in subgroups of lean (BMI <25 kg/m^2) and overweight/obese (BMI >25 kg/m^2) subjects. Based on computed tomographic scan measurement (L4 through L5) of abdominal visceral and subcutaneous fat areas, obese subjects were further categorized as predominantly viscerally or subcutaneously obese. *Predominantly visceral obesity* was defined as a ratio of visceral to subcutaneous fat area >0.5, as previously described [10]. Higher *TCF7L2* mRNA expression in subcutaneous as compared with visceral fat was confirmed in lean (5.5-fold) and in obese individuals (5.7-fold) (Fig. 1B) independent of fat distribution. No differences in expression were found between lean and obese subjects (Fig. 1B), and between subjects without and with T2DM (Fig. 1C).

Table 1
Anthropometric and metabolic characteristics of the study subjects

	NGT/IGT	T2DM
Male/female	18/19	5/7
Age (y)	64.5 ± 2.5	66.1 ± 2.3
BMI (kg/m^2)	29.7 ± 0.8	32.7 ± 1.9
Waist-hip ratio	1.03 ± 0.03	1.12 ± 0.03
Body fat (%)	32.1 ± 1.3	36.3 ± 3.0
Visceral fat area (cm^2)	141.4 ± 28.4 (n = 13)	236.9 ± 27.5 (n = 9)
SC fat area (cm^2)	328.2 ± 89.7 (n = 13)	384.3 ± 67.3 (n = 9)
HbA_{1c} (%)	5.5 ± 0.04	7.0 ± 0.13
Fasting blood glucose (mmol/L)	5.4 ± 0.07	7.0 ± 0.26
Fasting plasma insulin (pmol/L)	116.0 ± 14.5	257.2 ± 25.7
2-h OGTT glucose (mmol/L)	6.9 ± 0.2	15.2 ± 1.3 (n = 3)
Glucose infusion rate (mg/[kg min])	63.8 ± 4.2	27.3 ± 4.1 (n = 6)
FFA (mmol/L)	0.45 ± 0.05	0.82 ± 0.07
Total cholesterol (mmol/L)	5.2 ± 0.1	5.8 ± 0.3
HDL cholesterol (mmol/L)	1.4 ± 0.1	1.3 ± 0.1
LDL cholesterol (mmol/L)	2.9 ± 0.1	3.6 ± 0.2

Data are presented as means ± SEM. SC indicates subcutaneous; HbA_{1c}, hemoglobin A_{1c}; FFA, free fatty acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

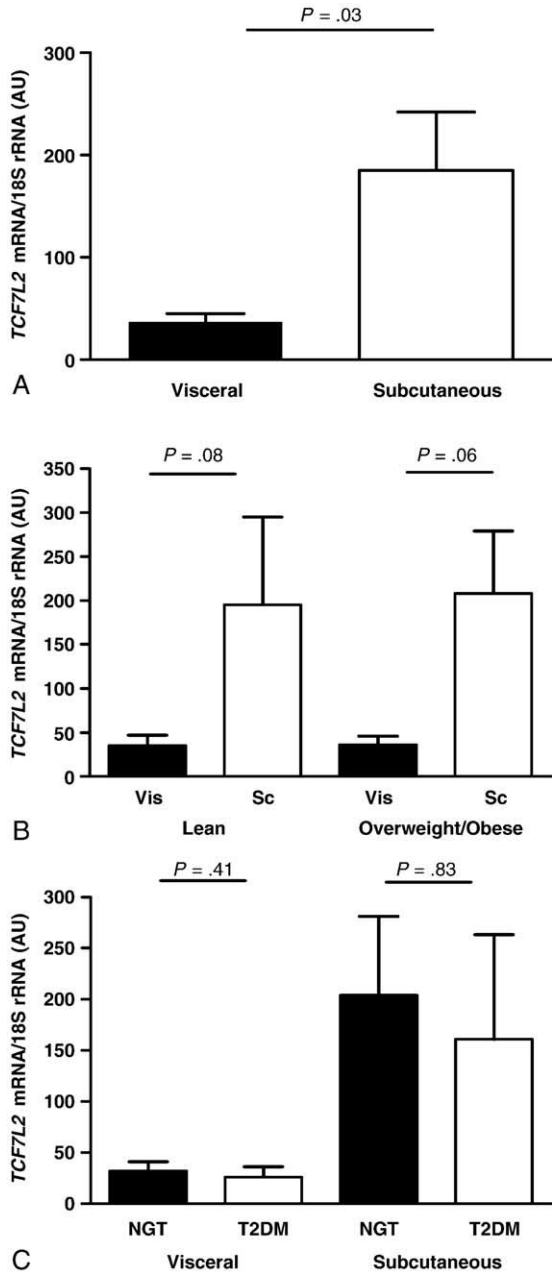

Fig. 1. *TCF7L2* mRNA expression in visceral and subcutaneous adipose tissue in lean, obese, NGT, IGT, and T2DM subjects. *TCF7L2* mRNA levels in (A) entire study population (N = 49), (B) subgroups of lean (n = 12) and overweight/obese subjects (n = 37), (C) subjects without T2DM (NGT, n = 33) or with T2DM (n = 12). Values are means ± SEM. Vis indicates visceral; Sc, subcutaneous; AU, arbitrary units.

TCF7L2 expression was also higher in subcutaneous than in visceral adipose tissue in subjects with (6-fold) or without (4.8-fold) T2DM (Fig. 1C). Furthermore, we did not find any correlation between *TCF7L2* mRNA levels and measures of obesity (BMI, waist-hip ratio, percentage body fat, visceral or subcutaneous fat area), glucose metabolism (fasting plasma glucose, 2-hour plasma glucose, fasting insulin), and lipid metabolism (total cholesterol, high- and low-density lipoprotein cholesterol) (data not shown). Correlation

between subcutaneous *TCF7L2* mRNA expression and glucose infusion rate in univariate regression analysis (R^2 = 0.071, P = .04) was abolished upon adjustment for age, sex, and BMI (P = .10).

3.2. TCF7L2 genetic variants and mRNA expression

No significant association of any of the previously reported SNPs [2] (rs7901695, rs7903146, rs12255372, rs11196205, and rs7895340) with visceral or subcutaneous mRNA levels was found (Supplemental table).

4. Discussion

Variants in the *TCF7L2* have been shown to be associated with an increased risk for T2DM in a number of population studies [4,6,11-14]. Most reports indicate that *TCF7L2* polymorphisms affect the capacity of pancreatic β-cells to secrete insulin rather than aggravating insulin resistance [4,6,12-16].

Expression of *TCF7L2* in human β-cells and adipose tissue has recently been reported [6]. In obese subjects with T2DM, *TCF7L2* expression was significantly decreased in different fat depots compared with obese normoglycemic individuals; and expression in subcutaneous adipose tissue was higher than in visceral fat [6]. We also observed higher subcutaneous than visceral *TCF7L2* mRNA levels; but there was no effect of age, sex, diabetes, and/or obesity on mRNA expression. It is unclear whether these regional differences in adipose tissue *TCF7L2* expression contribute to the causes of metabolic alterations associated with T2DM or represent their consequence. The latter seems to be more likely because no correlation between *TCF7L2* mRNA expression in both fat depots with metabolic traits related to obesity and T2DM was found in our study. However, based on the cross-sectional design of our study, where adipose tissue was collected from patients who had already been obese and/or developed T2DM, it seems difficult to decipher the causal inference, that is, whether the measured *TCF7L2* mRNA levels are secondary to obesity or T2DM, or vice versa. It is worth noting that, consistent with our findings, *TCF7L2* expression did not correlate with insulin sensitivity and BMI in a recent study including a Europid and African American sample [17].

We further tested the hypothesis that variability in adipose *TCF7L2* mRNA expression is caused by genetic variation in *TCF7L2*. Recently, Wang et al [16] showed that the T diabetes risk alleles of the rs12255372 and rs7903146 variants tended to be associated with lower *TCF7L2* expression in subcutaneous fat, whereas no relationships of these variants with adipocyte and muscle *TCF7L2* expression were observed by Elbein et al [17]. Although the known diabetes risk alleles of *TCF7L2* variants tended to be associated with lower subcutaneous expression also in our study, these effects did not reach statistical significance. Because *TCF7L2* interacts with the Wnt/β-catenin pathway and may hence mediate the inhibition of adipogenesis by tumor necrosis factor α [18], *TCF7L2* might contribute to the pathogenesis of obesity. However, *TCF7L2* variants do not appear to be involved in the genetic control of obesity. So far, no association or just a tendency for negative association with BMI has been reported for *TCF7L2* diabetes risk variants [4-6,13]. Our data suggest that *TCF7L2* expression in fat does not link obesity to the development of T2DM. We are aware that larger studies are necessary to confirm our findings because, owing to the limited sample size, we have been lacking statistical power to detect differences less than 50% in *TCF7L2* mRNA expression levels according to genotypic groups or the disease state. Nevertheless, the present study provides the largest collection of paired visceral and subcutaneous fat samples that has so far been reported. Furthermore, moderate associations of *TCF7L2* variants with some of the metabolic parameters (fasting blood glucose, fasting plasma insulin) were seen in our sample (data not shown); however, because of the very limited sample size, we see these associations with caution. Noteworthy, Elbein et al [17] found a trend toward genotype determination of gene expression in transformed lymphocytes. *TCF7L2* mRNA expression was decreased in nondiabetic individuals with the rs7903146 TT risk genotype, but increased in diabetic individuals with the TT genotype. This report suggests that tissue-specific *TCF7L2* splicing might partially explain the inconsistency seen in different tissues [17]. Although we did not address the potential effect of tissue-specific splicing on expression measures, a recent study by Cauchi et al [19] reported decreased *TCF7L2* expression in subcutaneous adipose tissue from normal glucose tolerance (NGT) obese rs7903146 TT carriers under calorie restriction; however, the polymorphism had no impact on the expression of specific *TCF7L2* isoforms with or without exon 4.

In conclusion, our data indicate fat-depot–specific *TCF7L2* mRNA expression, which however does not seem to be mechanistically linked to obesity or T2DM. The T2DM risk variants in the *TCF7L2* gene are not related to its mRNA expression in adipose tissue, indicating that *TCF7L2* expression or function in other organs/tissues may underlie the genetic association with T2DM.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.metabol.2008.04.016.

References

[1] Clatworthy JP, Subramanian V. Stem cells and the regulation of proliferation, differentiation and patterning in the intestinal epithelium: emerging insights from gene expression patterns, transgenic and gene ablation studies. Mech Dev 2001;101:3-9.

[2] Grant SFA, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7–like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320-3.

[3] Cauchi S, El Achhab Y, Choquet H, et al. *TCF7L2* is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. J Mol Med 2007;85:777-82.

[4] Florez JC, Jablonski KA, Bayley N, et al. *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 2006;355:241-50.

[5] Körner A, Berndt J, Stumvoll M, et al. *TCF7L2* gene polymorphisms confer an increased risk for early impairment of glucose metabolism and increased height in obese children. J Clin Endocrinol Metab 2007; 92:1956-60.

[6] Cauchi S, Meyre D, Dina C, et al. Transcription factor *TCF7L2* genetic study in the French population—expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes 2006;55:2903-8.

[7] Genuth S, Alberti KG, Bennett P, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160-7.

[8] Blüher M, Unger R, Rassoul F, et al. Relation between glycaemic control, hyperinsulinaemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or type II diabetes. Diabetologia 2002;45:210-6.

[9] Defronzo RA, Tobin JD, Andres R. Glucose clamp technique—method for quantifying insulin-secretion and resistance. Am J Physiol 1979;237:E214-23.

[10] Berndt J, Kloting N, Kralisch S, et al. Plasma visfatin concentrations and fat depot–specific mRNA expression in humans. Diabetes 2005; 54:2911-6.

[11] Kimber CH, Doney AS, Pearson ER, et al. *TCF7L2* in the Go-DARTS study: evidence for a gene dose effect on both diabetes susceptibility and control of glucose levels. Diabetologia 2007;50: 1186-91.

[12] Damcott CM, Pollin TI, Reinhart LJ, et al. Polymorphisms in the transcription factor 7–like 2 (*TCF7L2*) gene are associated with type 2 diabetes in the Amish—replication and evidence for a role in both insulin secretion and insulin resistance. Diabetes 2006;55: 2654-9.

[13] Saxena R, Gianniny L, Burtt NP, et al. Common single nucleotide polymorphisms in *TCF7L2* are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes 2006;55:2890-5.

[14] Scott LJ, Bonnycastle LL, Willer CJ, et al. Association of transcription factor 7–like 2 (*TCF7L2*) variants with type 2 diabetes in a Finnish sample. Diabetes 2006;55:2649-53.

[15] Munoz J, Lok KH, Gower BA, et al. Polymorphism in the transcription factor 7–like 2 (*TCF7L2*) gene is associated with reduced insulin secretion in nondiabetic women. Diabetes 2006;55: 3630-4.

[16] Wang J, Kuusisto J, Vanttinen M, et al. Variants of transcription factor 7–like 2 (*TCF7L2*) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia 2007; 50:1192-200.

[17] Elbein SC, Chu WS, Das SK, et al. Transcription factor 7–like 2 polymorphisms and type 2 diabetes, glucose homeostasis traits and gene expression in US participants of European and African descent. Diabetologia 2007;50:1621-30.

[18] Cawthorn WP, Heyd F, Hegyi K, et al. Tumour necrosis factor–alpha inhibits adipogenesis via a beta-catenin/TCF4(*TCF7L2*)-dependent pathway. Cell Death Differ 2007;147:1361-73.

[19] Cauchi S, Choquet H, Gutierrez-Aguilar R, et al. Effects of *TCF7L2* polymorphisms on obesity in European populations. Obesity 2008;16: 476-82.