**TCF7L2 rs12255372 and SLC30A8 rs13266634 Confer Susceptibility to Type 2 Diabetes in a Russian Population**

V. A. Potapov, M. N. Shamkalova, S. A. Smetanina, L. N. Bel'chikova, L. A. Suplotova, M. V. Shestakova, V. V. Nosikov

---

**Abstract**—Genes TCF7L2 and SLC30A8, encoding transcription factor-4 and transmembrane zinc transporter-8, respectively, play an important role in the regulation of development, proliferation, and pancreatic β-cell function. In the present study we examined polymorphic markers of genes rs12255372 [NT_03359.12:g33557428G>T] of TCF7L2 gene and rs13266634 [NP_776250.2:p.R325W] of SLC30A8 in groups of Russians with type 2 diabetes (T2D) (n = 588) and healthy normoglycemic controls (n = 597). Significant association of allele T (rs12255372) and allele R (rs13266634) with a higher risk of T2D development has been found (OR = 1.37 and 1.22, respectively). Adjustment for the effect of potential nongenetic risk factors resulted in a further increase in the OR values, from 1.54 (P = 0.24) to 1.89 (P = 0.046) for homozygous carriers of the T allele and from 1.29 (P = 0.035) to 1.35 (P = 0.019) in the individuals homozygous for the R allele. The patients homozygous for predisposing allele T (rs12255372) or R (rs13266634) had significantly lower insulin concentrations in the blood 2 h after glucose tolerance test (GTT) as well as lower values of HOMA-β, β cell homeostasis indicator compared to the carriers of other genotypes. Thus, we have shown that the rs12255372 and rs13266634 markers are independent genetic T2D risk factors in a Russian population.

**DOI:** 10.1134/S1022795410080132

---

**INTRODUCTION**

Type 2 diabetes (T2D) is the most common type of diabetes. The disease pathogenesis is determined by two major mechanisms: disturbance in insulin synthesis by pancreatic β-cells and development of insensitivity of peripheral tissue cells to insulin (insulin resistance syndrome). Recent genetic studies have revealed the number of gene polymorphisms associated with a high risk of T2D development. Moreover, the function of the majority of these “novel” predisposing genes is related to regulation of viability and proliferation of β-cells and their differentiation from precursors [1]. Among these genes TCF7L2 (transcription factor 7) and SLC30A8 (gene for transmembrane zinc transporter 8) play a key role in the development and function of pancreatic β-cells.

TCF7L2 gene encodes a transcription factor involved in WNT-signaling pathway, a major regulator of cell growth and development [2]. Introns 3 and 4 of the TCF7L2 gene contain single-nucleotide substitutions rs12255372 [NT_03359.12:g33557428G>T] and rs7903146 [NT_030059.12:g33506875C>T] shown to be associated with T2D [3]. Later, this association was confirmed in many populations of Caucasoids [4–10], Mongoloids [11–13], African Americans [14], and Caribbean Negroids [15]. In Caucasian populations, allele T of both markers was found to be associated with higher risk of T2D development (Odds Ratio OR reaches 1.40) [6, 16]. In addition, the carriers of predisposing allele possessed numerous defects in insulin secretion system in β-cells resulting in decreased insulin outflux into the blood flow in response to glucose stimulation [17], decreased rate of proinsulin into insulin conversion [18], as well as accumulation of inactive prohormone (proinsulin) associated with decreased response of peripheral tissues to insulin [19].

The involvement of the SLC30A8 gene in the T2D development was shown in several large-scale studies searching for genomic associations [20–25]. The strongest association with diabetes was observed for polymorphic mononucleotide substitution rs13266634 [NP_776250.2:p.R325W] in exon 8 resulting in arginine (R) substitution for tryptophan (W) (OR = 1.12 in Caucasoids). The SLC30A8 gene encodes transmembrane zinc transporter-8 (ZnT-8) located in the secretory granules of β-cells and respon-
sible for active transport of Zn\(^{2+}\) ions into the insulin granules. Zink plays an important role in regulation of maturation, storage, and efflux of insulin by β cells [26]. The carriers of the predisposing allele R325 decreased insulin secretion in response to stimulation by glucose and a decreased rate of conversion of proinsulin into insulin [19].

In spite of the fact that association of TCF7L2 and SLC30A8 genes with T2D has been shown in a number of ethnic groups, the role of these genes in the etiology of the disease in the Russian population has not been studied so far. Russia ranks fifth in the world in T2D patient number (9.6 million in 2007) [28]. Therefore, we considered important to reveal whether these genetic markers are associated with the risk of T2D development in the Russian population. Here, we show a significant association between marker rs12255372 of the TCF7L2 gene and rs13266634 of the SLC30A8 gene with T2D development in the Russian population.

**MATERIALS AND METHODS**

**Patients**

We examined 1185 unrelated Russians from Moscow aged over 50, including 588 T2D patients and 597 healthy normoglycemic individuals (control group). For further biochemical and genetic analyses, the blood samples were taken in the Endocrinological Research Center, Russian Academy of Medical Sciences (402 T2D patients and all the healthy individuals) and in Tyumen State Medical Academy of Federal Health and Social Development Agency (186 T2D patients). T2D was diagnosed according to the WHO criteria: blood plasma glucose concentration after fasting ≥7.0 mM, or glucose concentration in the blood plasma ≥11.1 mM 2 h after glucose tolerance test (TTG), i.e., peroral administration of 75 g glucose or drugs decreasing glucose concentration in the blood [29].

The control sample consisted of individuals with normal glucose tolerance, the level of glycosylated hemoglobin (HbA1c) < 6.1%, and without family history of T2D. The patients with diabetes type 1, gestational diabetes, and monogenic forms of diabetes as well as the individuals with clinical symptoms of secondary diabetes resulting from pancreatitis, hemochromatosis, and other diseases were excluded from the study. Blood pressure was measured using tonometer. Hypertension was diagnosed at systolic pressure >140 mm Hg, diastolic pressure > 90 mm Hg or if hypertension was indicated in the disease history. The patients with body mass index (BMI) over 30 kg/m\(^2\) were considered obese [30].

**Biochemical Analysis**

Plasma fasting concentration of cholesterol, complexes of high density lipoprotein (HDL) with cholesterol, triglycerides and glucose were measured using standard enzymatic methods. The amount of complexes of low density lipoprotein (LDL) with cholesterol were calculated using Friedewald equation [31]. Standard peroral TTG test was conducted after 12 h of fasting in accordance to WHO recommendations. HbA1c levels were determined by ion exchange high performance liquid chromatography (HPLC) (reference interval for healthy individuals was 4.0—6.1%). Insulin concentration in blood plasma was determined using immune enzyme analysis. HOMA-β index, estimating of β cell function according to the homeostatic model, was calculated as

\[
\text{HOMA-β} = 20 \times \left( \frac{\text{insulin concentration after fasting, } \mu\text{U/l}}{\text{glucose concentration after fasting, } \text{mM}} \right) - 3.5.
\]

According to the homeostatic model HOMA-IR insulin resistance index was estimated as

\[
\text{HOMA-IR} = \frac{\text{insulin concentration after fasting, } \mu\text{U/l}}{\text{glucose concentration after fasting, } \text{mM}}/22.5 [32].
\]

Clinical and metabolic data on T2D patients and control group are presented in Table 1.

**DNA Analysis**

Genomic DNA was isolated from peripheral blood using Genome DNA Purification Kit (Fermentas, Lithuania) according to the manufacturer’s recommendations. The rs12255372 (TCF7L2) and rs13266634 (SLC30A8) markers were genotyped by direct sequencing of polymerase chain reaction (PCR) products containing the polymorphic site, using a BigDye terminator and automated DNA sequencer ABI PRISM 3100-Avant (Applied Biosystems, United States) according to the manufacturer’s recommendations. The PCR products were amplified using a PTC-225 Thermal Cycler (MG Research, United States) and the following program: initial denaturation, 95°C/2 min, 30 cycles at 95°C/20s, 60°C/20s, 72°C/30s followed by final elongation at 72°C/5min. The reaction mixture (20 μl) contained 75 mM Tris-HCl (pH 8.8), 20 mM (NH\(_4\))\(_2\)SO\(_4\), 1.0 mM MgCl\(_2\), 0.2 mM of each dNTP, 0.01% Tween 20, 10 ng genomic DNA, 0.5 U Taq DNA polymerase (Fermentas, Lithuania), and 10 pg of the forward and reverse primers. For amplification of TCF7L2 gene region containing rs12255372 marker the primers 5’-CAACTGGGATTTCAAGATGTCCCT-3’ (forward) and 5’-CTGCTATTGACGCTTACAAATG-3’ (reverse) were applied. In case of the rs13266634 marker, the following primers were used: 5’-CAAACTGTCAGTCTTCCTCTAGA-3’ (forward) and 5’-TCCTGGTCATCGCACATGATTCC-3’ (reverse). Prior to the sequencing the PCR products were purified of excess of enzyme, primer, and dNTP using GeneJET™ PCR Purification Kit (Fermentas, Lithuania).