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Original article

Relationship of transcription factor 7 like 2 gene rs7903146 variation with type 2 diabetes and obesity related parameters

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ABSTRACT

Aims: The allele frequencies of transcription factor 7 like 2 (TCF7L2) gene rs7903146 polymorphism in type 2 diabetes mellitus (T2DM) and non-T2DM controls were determined.
Methods: TCF7L2 rs7903146 genotypes were determined with qPCR.
Results: The TCF7L2 gene rs7903146 genotype frequencies for homozygous wild type (C/C), heterozygous (C/T) and homozygous polymorphic (T/T) for T2DM patients were determined, respectively, as 71.4%, 14.3%, 14.3% and 72.5%, 11.8%, 15.7% for controls. The weight, length and lean body mass were higher in C/T + T/T compared to C/C carriers. Glucose, insulin, insulin resistance and homeostatic model assessment (HOMA) were nonsignificantly higher in rs7903146C/T + T/T in comparison to C/C. TCF7L2 gene rs7903146 genotypes were not found to interact with drugs. The absence of any difference between genotype frequencies among study groups indicates that no association persists with TCF7L2 gene rs7903146 polymorphism and T2DM.
Conclusions: The effects of rs7903146 variation over some obesity variables suggest that this variation may effect T2DM development via obesity.

1. Introduction

Transcription factor 7-like 2 (TCF7L2) gene is located on chromosome 10q25.3 (114700201–114916063, NCBI build 36.2) [1]. TCF7L2 has been revealed as an susceptibility gene for a type 2 diabetes by DECODE group. Other many studies have stated TCF7L2 gene to be associated with type 2 diabetes [2].

TCF7L2 gene codes a transcription factor with a high-mobility box and functions in gene activations related to downstream events of the Wnt signaling pathway and T2DM [3–6]. The NCBI Refseq for TCF7L2 contains 14 exons whereas another study has reported TCF7L2 to consist of 17 exons, with five of them as alternatives [7].

A minimum of four polymorphic markers are subject to interest which are rs7903146: C-to-T (position: 114748339) substitution within intron 3 [2,4,8]; rs7901695: T-to-C (position: 114744078) substitution within intron 3 [2,8,9]; rs12255372: G-to-T (position: 14798892) substitution within intron 4 [2,10]; rs11196205: G-to-C (position: 114797037) substitution within intron 4 [11,12]. The single nucleotide polymorphisms (SNPs) identified by Grant et al. [2], have been extensively examined by other investigators within different ethnic groups [11,13–19], and among them, rs12255372 and rs7903146 are most strongly associated with type 2 diabetes [2,4,13,16,20,21].

TCF7L2 has been reported to be widely expressed in mature pancreatic beta cells, peripheral tissues and adipocytes of omentum [13]. The WNT pathway has regulatory roles in adipogenesis [22]; thus, a major physiological role of TCF7L2 in glucose homeostasis may be hypothesized. TCF7L2 gene rs7903146 risk allele has been found to reduce insulin secretion as a response to oral glucose intake [22,23]. In Europid population, a significant association was detected to reduce insulin sensitivity index (SI) and the beta cell compensation for insulin resistance in variant rs7903146 genotype [24,25]. In contrast, Scott et al. [11] were unable to report any association with fasting insulin, glucose tolerance, SI, whereas Cauchi et al. [13] found non-significantly reducing fasting insulin and HOMA.

We examined the allele frequencies of transcription factor 7 like 2 (TCF7L2) gene rs7903146 polymorphism in T2DM and

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non-T2DM subjects and detected the effects of rs7903146 genotypes on anthropometric, diabetes and obesity related parameters. We also searched for independent effects of T2DM risk factors together with rs7903146 genotype effect on disease by logistic regression analysis. The pharmacogenomic effects of rs7903146 polymorphism on hypertension and type 2 diabetes drugs were also evaluated.

2. Subjects

We studied 56 T2DM patients (28 men, 28 women), 51 controls (30 men, 21 women), from Turkish Diabetes and Obesity Foundation Hospital (Istanbul, Turkey). Type 2 diabetic patients were selected according to WHO criteria [26]. T2DM was fasting blood glucose ≥126 mg/dl, 2-h postload glucose 200 mg/dl during an OGTT. The test was performed, as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

Other disease such as obesity, hypertension and dyslipidemia were diagnosed according to International Diabetes Federation (IDF) guidelines [27]. The mean age was 51.08 ± 2.12 for T2DM patients and 54.57 ± 1.40 for controls. Written consent was obtained from each patient following a full explanation of the study, which has been approved by the Ethics Committee of the Marmara University.

3. Materials and methods

3.1. Analytical methods

3.1.1. Pancreatic β-cell secretory capacity

Pancreatic β-cell secretory capacity was calculated by the β-cell index (index of β-cell secretory force, HOMA β-cell index) using the below formula [28]:

$$\text{HOMA}\beta\text{-cell index} = 20 \times \frac{I^{F}}{G^{F} - 3.5}$$

where I^{F} is the fasting insulin (in μU/ml) and G^{F} is the fasting plasma glucose (in mg/dl).

3.1.2. The rate of insulin resistance

The rate of insulin resistance was evaluated by the homeostatic model assessment [29] and calculated by the formula below [30]:

$$\text{Insulin resistance (IR)} = \frac{\text{fasting serum insulin (in mU/ml)} \times \text{fasting plasma glucose (in mmol/l)}}{22.5}$$

3.1.3. Hepatic insulin sensitivity

Hepatic insulin sensitivity was calculated using the formula described below [31]:

$$\text{hepatic insulin sensitivity (HIS)} = \frac{k}{G^{F}} \text{ (in mg/dl)} \times I^{F}\text{(in mU/ml)}$$

$$\text{where } k = 22.5 \times 18 = 405$$

3.1.4. Body fat quantification

Lean body mass (LBM) was calculated as [32]: for males, LBM (kg): 0.32810 × weight (in kg) + 0.33929 × height (in cm) − 29.5336; for females (kg): 0.29569 × weight (in kg) + 0.41893 height (in cm) − 43.2933. Body fat was calculated by subtracting the lean body mass from the present body weight.

3.1.5. Biochemical measurements

The biochemical analyses included determination of fasting plasma glucose, triglyceride (TG), total-cholesterol (T-Chol), high-density lipoprotein-cholesterol (HDL-Chol), low-density lipoprotein-cholesterol (LDL-Chol). Serum TG and T-Chol levels were measured using standard enzymatic methods (Merck, Darmstadt, Germany), on an AU5021 device (Olympus, Merck). Serum HDL-Chol and LDL-Chol were determined by immunonephelometry on a Behring Nephelometer analyzer with Behring reagents (Behringwerke, Marburg, Germany).

3.1.6. DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard salting out method [33] and High Pure PCR Template Preparation Kit, Roche. Purified DNA (concentration of 50 ng) was stored at −20 °C. The rs7903146 polymorphism was determined by Real-Time Polymerase Chain Reaction analysis using the LightCycler 2.0 Instrument (Roche Diagnostics, Germany). LightCycler® FastStart DNA Master HybProbe kit was used to detect TCF7L2 gene rs7903146 genotypes. The resulting PCR fragments were analyzed with hybridization probes labeled with Simple Probe (TıbMolBiol LightSNip Kit) (TIBMOLBIOL, Germany) (detected in channel 640). The genotypes were identified by running a melting curve with specific melting points (T_m). The wildtype human TCF7L2 rs7903146 DNA exhibits a T_m of 57.95 °C in channel 640. The polymorphic human TCF7L2 rs7903146 exhibits a T_m of 51.64 °C in channel 640. The supplied control DNA allows the accurate comparison with unknown samples. The control DNA has been prepared with PCR grade water, 10^5 target molecules per reaction. Each PCR run contained a negative (no template) control. After 10 min denaturation at 95 °C, the amplification was performed by running 45 cycles 10 s at 95 °C, 10 s at 60 °C and 15 s at 72 °C. The melting program included three steps: denaturalization at 95 °C for 30 s, renaturation at 40 °C for 2 min, and then slowly raised to 75 °C to allow monitoring of the decline of fluorescence generated by melting of the hybrids, as a function of temperature. Then subsequent cooling step was performed at 40 °C for 30 s. Melting curves were automatically converted to fluorescence peaks with the LightCycler 2.0 Instrument analysis software, allowing the distinction of genotypes. The grouping software uses a curve shape-matching algorithm to identify wild type from polymorphic samples and cut-offs are based on variability from the wild type curve. The results were confirmed for homozygous polymorphic, homozygous wild type and heterozygous samples in all repeated measurements.

3.1.7. Statistical analysis

Statistical analyses were calculated with the SPSS 17.0 software. Data were given as mean ± SE. Baseline differences between patients and controls were examined by Student's *t*-test. Mann–Whitney *U* equilibrium for genotype frequencies was estimated by the Chi-square and Fisher test. Sex, fat mass, body mass index, dyslipidemia, fasting plasma glucose, waist circumference and the rs7903146 genotypes of the TCF7L2 gene were selected as potential risk factors for type 2 diabetes. The predictors of T2DM were determined by stepwise logistic regression analysis. Odds ratios (OR) with two tailed *p* values were calculated as a measure of the association of the selected parameters with essential T2DM. *p*-Values less than 0.05 were considered significant.

4. Results

The frequencies of the human TCF7L2 gene rs7903146 genotypes were given in Table 1. The TCF7L2 gene rs7903146 genotype frequencies for homozygous wild type (C/C), heterozygous (C/T) and homozygous polymorphic (T/T) T2DM patients