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Research paper

The effects of TLR3, TRIF and TRAF3 SNPs and interactions with environmental factors on type 2 diabetes mellitus and vascular complications in a Han Chinese population

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ABSTRACT

Toll-like receptor 3 (TLR3) is involved in type I interferon-β (IFN-β) via TIR-domain-containing adapter-inducing interferon-β (TRIF) and Tumor necrosis factor receptor–associated factor 3 (TRAF3), culminating in inflammation and immunity reactions. TLR3 is implicated in insulin resistance and type 2 diabetes mellitus (T2DM). Eight SNPs of these genes were detected in 552 T2DM patients and 552 matched healthy control subjects. Gene-gene and gene-environment interactions and haplotype associations were also evaluated. We identified a 21% increased risk of T2DM for the T allele of rs12435483 in the TRAF3 gene (OR: 1.21; 95% CI: 1.01–1.44; $P = 0.036$). The GA genotype and GA + AA genotype of TRAF3 rs12147254 were found to increase the risk of coronary heart disease (CHD) among T2DM patients (GA vs. GG: OR = 4.17, 95% CI: 1.04–16.79, $P = 0.045$; GA + AA vs. GG: OR = 3.97, 95% CI: 1.02–15.48, $P = 0.047$). However, the GACGAC haplotype in TRAF3 had a protective effect on T2DM micro-macrovascular complications (OR = 0.33, 95% CI: 0.13–0.85, $P = 0.017$). Two-factor (TRAF3 rs12435483 and LDL) and three-factor (TRAF3 rs12435483, BMI and HDL) interactions of the risk of T2DM were identified. In conclusion, the genetic variants in the TLR3-TRIF-TRAF3-INF-β signaling pathway and interactions with some particular environmental factors (LDL, BMI and HDL) may contribute to susceptibility to T2DM and vascular complications in the Han Chinese population.

1. Introduction

The International Diabetes Federation (IDF) reported that the number of people worldwide with diabetes mellitus would increase from 415 million in 2015 to 642 million in 2040 with 5.0 million deaths globally (IDF, 2015). Greater than 95 percent of these adult patients have type 2 diabetes mellitus (T2DM) (IDF, 2015). T2DM is characterized by damages to insulin secretion and sensitivity, which result in hyperglycemia (Stumvoll et al., 2005). Hyperglycemia promotes the occurrence of T2DM micro-macrovascular complications, such as cerebral infarction (DCI) and coronary heart disease (CHD) (Lontchi-Yimagou et al., 2013). Large clinical studies have certified the crucial importance of diabetes mellitus control for preventing vascular complications and improving the quality of life and longevity (Hayward et al., 2015). Complications can lead to heart attack, stroke, blindness, kidney failure and lower limb amputation (Hayward et al., 2015). There

Abbreviations: TLR3, toll-like receptor 3; TRIF, TIR-domain-containing adapter-inducing interferon-β; TRAF3, tumor necrosis factor receptor–associated factor 3; IFN-β, type I interferon –β; NF-κB, nuclear factor-κB; IRF-3/7, interferon regulatory factor 3/7; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; SD, standard deviation; DR, diabetic retinopathy; DN, diabetic nephropathy; DPN, diabetic peripheral neuropathy; DCI, diabetes cerebral infarction; CHD, coronary heart disease; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; UTR, untranslated region; MDR, multifactor dimensionality reduction; KCNQ1, potassium voltage-gated channel subfamily Q member 1; TLR2, toll-like receptor 2

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were 109.6 million Chinese diabetes patients in 2015 (IDF, 2015). The economic burden was heavy for direct medical costs of diabetes in China, accounting for 18% of national total health expenses in 2007, which is far greater than the international level (Zheng et al., 2012).

Several studies have demonstrated that a series of inflammatory responses are particularly associated with the pathogenesis of T2DM (Donath and Shoelson, 2011; Lontchi-Yimagou et al., 2013) and T2DM vascular complications (Lontchi-Yimagou et al., 2013). Toll-like receptor 3 (TLR3), which is highly and broadly expressed in pancreatic β-cells, binds to dsRNA and is activated. Then, activated TLR3 accelerates β-cell dysfunction and apoptosis, resulting in insulitis and autoimmunity (Strodthoff et al., 2015). TLR3 signals are involved in activating type I interferon-β (IFN-β) via TIR-domain-containing adapter-inducing interferon-β (TRIF) and tumor necrosis factor receptor–associated factor 3 (TRAF3) (Takeda and Akira, 2004). TLR3-deficient mice with obesity exhibit enhanced glycemic control facilitated by elevated insulin secretion, indicating that TLR3 played a key role in metabolic regulation (Strodthoff et al., 2015). TLR3 gene deficiency protected obese mice against insulin resistance and reduced liver steatosis (Wu et al., 2012). Notably, overexpression of TRIF activates the IFN-β promoter (Takeda and Akira, 2004). TLR3-associated gene expression of IFN-β was altered in TRIF knockout mice (Yamamoto et al., 2003). TRIF-mutant mice were deficient in TLR3-mediated responses, and TRIF-deficient mice were protected against lard-induce white adipose tissue inflammation and impaired insulin sensitivity (Caesar et al., 2015). In addition, TRIF deficiency is associated with hyperglycemia and β-cell dysfunction, and the TLR pathway via TRIF is important for the normal function of β-cells and glucose tolerance (Hutton et al., 2010). TRAF3 plays a crucial role in TRIF adaptor, and cells lacking TRAF3 are defective in IFN responses mediated by TLR3/4 (Hacker et al., 2006). Furthermore, TRAF3 binds to TRIF family member-associated nuclear factor (NF)-κB activator (TANK) and NAK-associated protein 1 (NAP1), a TANK homologue, to activate interferon regulatory factor 3/7 (IRF-3/7) kinases TBK1 and IKK-ε. These results indicate that TRAF3 could serve as downstream regulatory kinase in the type I IFN pathway (Hacker et al., 2006). Hepatocyte TRAF3 induced insulin resistance and T2DM in obese mice, whereas myeloid TRAF3 increased metabolic inflammation, insulin resistance, and hepatic steatosis in obesity (Na et al., 2015).

Variations in TLR genes exhibit crucial importance in the pathophysiology of inflammatory diseases, and single nucleotide polymorphisms (SNPs) of TLR genes are associated with T2DM and complications (Bagarolli et al., 2010). For instance, the Asp299Gly polymorphism in TLR4 increases the risk of cardiovascular disease complications in T2DM patients (Buraczynska et al., 2016). Five SNPs in TLR3 were related to non-healing diabetic foot ulcers in T2DM patients (Singh et al., 2013), whereas TLR3 rs3775291 was genetically related to elevated fasting insulin levels (Strodthoff et al., 2015). However, it remains unknown whether polymorphisms in the TLR3-TRIF-TRAF3-INF-β pathway contribute to T2DM and vascular complications. Elucidating the correlations among T2DM and vascular complications and eight highly associated SNPs in TLR3, TRIF and TRAF3 is crucial to evaluate the probability of related genetic polymorphisms in predicting the risk of T2DM and vascular complications in the Han Chinese population.

2. Methods and materials

2.1. Study subjects

A total of 1104 Chinese Han residents in Guangzhou were included in this study. Blood samples from patients with T2DM and healthy control volunteers were obtained from the Overseas Chinese Hospital in Guangzhou with informed consent from September 2011 to January 2013. This study was approved by the ethics committee of School of Medicine in Jinan University, China. In total, 552 randomly chosen controls with normal glucose tolerance and without T2DM family history were matched with 552 randomly chosen T2DM cases based on age (± 5 years old) and gender. All T2DM patients were diagnosed by the 2003 American Diabetes Association criteria (Genuth et al., 2003). Patients with type 1 diabetes (T1DM) and abnormal glucose tolerance tests were excluded from the study. Among T2DM patients, 128 cases were diagnosed with T2DM without any complications, whereas 221, 63 and 71 patients had microvascular complications, macrovascular complications and micro-macrovascular complications, respectively. Microvascular complications include diabetic retinopathy (DR), diabetic nephropathy (DN) and diabetic peripheral neuropathy (DPN), whereas macrovascular complications include coronary heart disease (CHD) and cerebral infarction (DCI). Micro-macrovascular complications include both microvascular and macrovascular complications.

2.2. SNP selection

We used the Hapmap database (http://hapmap.ncbi.nlm.nih.gov) to choose crucial tagSNPs according to the genetic information from Han Chinese in Beijing (CHB) population by setting the Hardy-Weinberg *P*-value > 0.01, minor allele frequency (MAF) > 0.05 and $r^2 \geq 0.8$. Eight SNPs were identified, including rs3775291 in the TLR3 gene; rs8120 in the TRIF gene; rs12435483, rs7156191, rs3803286, rs12588538, rs2144826 and rs12147254 in the TRAF3 gene. General information regarding these SNPs, including gene locations, alleles, MAFs, Hardy-Weinberg *P*-values and call rates, is described in Table S.1. Three SNP (rs8120, rs12588538 and rs12147254) did not meet Hardy-Weinberg equilibrium (HWE) ($P < 0.05$), so these SNPs were excluded when we analyzed the polymorphism associations between T2DM cases and health controls. All eight SNPs were further analyzed to assess the association between T2DM and T2DM vascular complications.

2.3. DNA extraction and genotyping

Genomic DNA was extracted from peripheral whole blood samples by QIAamp Blood DNA Mini Kit (Qiagen, Hilden, Germany), and we genotyped all 8 SNPs of participants using the Sequenom MassARRAYiPLEX Gold analyzer (Sequenom, Life Technologies, Shanghai). Polymerase chain reaction (PCR) conditions and primers were designed by MassARRAY Assay Design 3.1 software (see Table S.2).

2.4. Quantitative real-time reverse transcription PCR (qRT-PCR) analyses

Total RNA was isolated from peripheral whole blood samples using Blood RNA Kit (Omega Bio-Tek, Doraville, GA, USA), followed by reverse transcription using transcriptase cDNA kit (TakaraPrimeScript RT Master Mix kit, Otsu, Japan). Then qRT-PCR analysis was performed to quantify mRNA expression of TRAF3 with SYBR PrimerScript RT-PCR kit (Takara, Otsu, Japan) normalized to mRNA β-actin, and Bio-Rad CFX96 realtime system (Bio-Rad Laboratories) was used to perform the assays. Cycle conditions were 95 °C for 30s and 45 cycles consisting of 95 °C for 5 s and 60 °C for 30s. The $2^{-\Delta\Delta Ct}$ method was used to calculate mRNA relative expression level. Data was analyzed using two-tailed Mann-Whitney test.

2.5. Statistical analyses

Haploview software version 4.2 and SHEsis (Yong and Lin, 2005) were used to select haplotype blocks and evaluate haplotype effects in the TRAF3 gene as described by Li Z et al. (Li et al., 2009). All calculations were conducted by Statistical Product and Service Solutions software (SPSS 13.0) (IBM Corp, Armonk, NY, USA). *P*-value < 0.05 was considered statistically significant. Chi-square test (χ^2 test)