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Association of polymorphisms in the MyD88, IRAK4 and TRAF6 genes and susceptibility to type 2 diabetes mellitus and diabetic nephropathy in a southern Han Chinese population

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ABSTRACT

Type 2 diabetes mellitus (T2DM) has been linked to a state of low-grade inflammation resulting from abnormalities in the innate immune pathway. MyD88 is an essential adaptor protein for TLR signaling, which is involved in activating NF-κB through IRAK4 and TRAF6. To investigate the effects of the MyD88, IRAK4 and TRAF6 polymorphisms in the susceptibility of T2DM and diabetic vascular complications, eight SNPs were analyzed in 553 T2DM patients and 553 matched healthy controls. Gene-gene interactions and haplotype associations were also evaluated. We found a significant increased risk of T2DM for the AG genotype of rs6853 in MyD88 gene and the CT genotype of rs4251532 in IRAK4 gene. Significant association was also found between rs16928973 in TRAF6 gene and diabetic nephropathy (DN) under the allelic model. Moreover, the TA haplotype in TRAF6 was negatively associated with DN. No significant gene-gene interactions were found. In conclusion, our results indicate that the polymorphisms in TLR-MyD88-NF-κB signaling pathway confer genetic susceptibility to T2DM and DN.
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1. Introduction

The International Diabetes Federation estimated that 382 million people suffered from diabetes, and the number of patients with diabetes is predicted to rise to 592 million in 2035 (Federation, 2013). Type 2 diabetes mellitus (T2DM) is the most common type. However, many people with T2DM are often diagnosed only when diabetic complications have already developed. The increasing incidence of T2DM and the importance of early diagnosis press for findings to identify environmental and genetic factors that contribute to the susceptibility of T2DM and its complications.

T2DM is a complex endocrine and metabolic disorder, caused by impaired glucose tolerance (IGT) as a result of insulin resistance and consequential islet β-cell exhaustion, with ensuing insulin deficiency impacting skeletal muscle, liver and adipose tissues (Stumvoll et al., 2005). And inflammation has been suggested to be linked with the pathogenesis of T2DM (Shoelson et al., 2006). Inflammatory response is most likely to account for the occurrence of T2DM by causing insulin resistance, and is, in turn, intensified in the presence of hyperglycemia to promote both microvascular and macrovascular complications, such as diabetic retinopathy (DR), diabetic nephropathy (DN) and diabetic peripheral neuropathy (DPN) (Lontchi-Yimagou et al., 2013).

Several studies have identified the increased levels of both circulating and cellular biomarkers of inflammation in diabetics, especially the toll-like receptors (TLRs) (Jialal and Kaur, 2012). Myeloid differentiation factor 88 (MyD88) is an essential adaptor

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protein, which is mediated by all TLR members signal transduction, except for TLR3 (Li et al., 2009a). Upon ligands binding to TLR, the adaptor molecule MyD88 is recruited to TLR complex as a dimmer. Then MyD88 recruits interleukin-1 receptor (IL-1R) associated kinase 1 (IRAK1), IL-1R associated kinase 4 (IRAK4), and tumor necrosis factor receptor associated factor 6 (TRAF6) (Akira and Takeda, 2004), which results in the activation of nuclear factor-kappa B (NF-κB) and production of inflammatory cytokines such as IL-1α, IL-1β, tumor necrosis factor (TNF)-α, and IL-6 (Christman et al., 2000). NF-κB, TNF-α, IL1 and IL6 are vital in the development of insulin resistance (Patel and Santani, 2009). Dasu MR et al. (Dasu et al., 2010) has showed that the serum concentration of IL-1β, IL-6, and TNF-α and the expression level of MyD88, phosphorylated IRAK-1, TRAF, and NF-κB p65 in monocytes in T2DM patients increased significantly compared with control subjects. Jialal I et al. (Jialal and Pahwa, 2015) also summarized the evidence about the role of TLRs in diabetic vascular complications especially DN. Diabetic rat kidneys have shown increased protein and mRNA expression of TLR-2, MyD88 and NF-κB (Li et al., 2010). Recent study by Jialal I et al. (Jialal et al., 2014) showed that DN is also mediated by TLR-4 in TLR-4 knockout mice.

Genetic variations within genes encoding TLRs have an important influence on the pathogenesis of inflammatory diseases (Schröder and Schumann, 2005). Recent studies have highlighted the role of single nucleotide polymorphisms (SNPs) of TLR genes in the presence of T2DM. For example, the genotypes of SNPs rs4986790 and rs4986791 in the TLR4 gene were associated with protection of T2DM (Bagarolli et al., 2010; Manolakis et al., 2011), the genotypes of SNPs rs4986790, rs4986791, rs11536858, rs1927911, and rs1927914 in TLR4 gene individually or in combination may impair the wound healing process in T2DM patients resulting in non-healing diabetic foot ulcer (DFU) (Singh et al., 2013), and the G allele of rs4986790 was an independent risk factor of early onset DN in the T2DM patients (Buraczynska et al., 2009). The SNPs in TLR-MyD88-NF-κB signaling pathway adaptor molecules which include MyD88, IRAK4 and TRAF6 have been reported to be correlated with infections diseases (Carrasco-Colom et al., 2015), but, to date, little is known about the association between these SNPs and diabetes. Considering this, we decided to investigate the association between polymorphisms in the MyD88, IRAK4 and TRAF6 genes and T2DM and its vascular complications in Han Chinese population from Guangzhou.

2. Methods

2.1. Study subjects

A total of 1106 subjects of Southern Han Chinese ancestry residing in Guangzhou were included in the study. All patients with T2DM were hospitalized in the Endocrinology Departments of Overseas Chinese Hospital in Guangzhou from September 2011 to January 2013. 553 control subjects were volunteers who had normal glucose tolerance and no family history of T2DM, matched with T2DM patients by gender and age (±5 years). T2DM was defined as fasting blood glucose equal or more than 7.00 mmol/L or 2 h plasma glucose equal or more than 11.0 mmol/L during an oral glucose tolerance test with diabetes clinical symptoms. We excluded patients with type 1 diabetes and other abnormal glucose tolerance. DR was diagnosed by independent ophthalmologists. Ophthalmoscopic examination through dilated pupils and fluorescein angiography were conducted. DN was determined on the basis of 24-h albumin excretion rate (AER) and estimated glomerular filtration rate (eGFR). The DN was diagnosed in the presence of overt albuminuria (AER > 300 mg/24 h) and eGFR < 60 mL/min/1.73 m^2, in the absence of any clinical or laboratory evidence of other renal disease. The diagnosis of DPN was according to the standard diabetic neuropathy symptom score and diabetic neuropathy disability score criteria (Dyck et al., 1995; Young et al., 1993). Coronary heart disease (CHD) was diagnosed by the presence of stenosis of more than 50% luminal diameter in at least one significant coronary artery on coronary angiography (Patel et al., 2014). Hypertension (DH) was defined as a diastolic blood pressure ≥ 90 mmHg and/or a systolic blood pressure ≥ 140 mmHg and/or the use of anti-hypertension medication (Chalmers, 1999). The diagnosis of cerebral infraction (DCI) was based on brain magnetic resonance imaging (MRI), magnetic resonance angiography (MRA) and cardiac color ultrasound. Among these patients, 207, 87, 137, 80, 317 and 65 were diagnosed with DR, DN, DPN, CHD, DH and DCI, respectively and 87 without any complication. This study was approved by the ethics committee of School of Medicine in Jinan University, China and performed strictly in accordance with the Declaration of Helsinki. All participants gave written informed consent before entry into this study.

2.2. SNP selection

The tagSNPs were chosen from the Hapmap database (http://hapmap.ncbi.nlm.nih.gov/) according to the following selection strategy. Firstly, the screened region extended 10 kilobases upstream of the annotated transcription start site and downstream at the end of the last each gene exon, which cover most of the genetic information in the Han Chinese in Beijing (CHB) population from the HapMap database (HapMap data rel 27 Phase II + III, Feb2009) (Consortium, 2005). 21 tagSNPs were obtained and loaded in the Haploview software version 4.2 (Broad Institute) (Barrett et al., 2005). Secondly, tagSNPs were then selected using a pairwise tagging algorithm setting the Hardy-Weinberg P-value, minor allele frequency (MAF) and r^2 thresholds at 0.01, 0.05 and 0.8, respectively. The linkage disequilibrium (LD) pattern of each gene in the CHB population exhibited strong LD in several groups of tagSNPs ($r^2 \geq 0.8$), indicating that most common SNPs can be captured by a subset of tagging SNPs. We selected 8 SNPs, including rs7744, rs6853 in MyD88 gene, rs4251569, rs1461567, rs4251513 and rs4251532 in IRAK4 gene, and rs16928973, rs5030445 in TRAF6 gene. The genotype distributions of the 8 SNPs with gene locations, alleles, MAFs, Hardy-Weinberg P-values and call rates were showed in Table S1. The overall call rates were from 94.9% to 100% in all samples. The MAFs ranged from 3.1% to 45.1% and the observed genotype frequencies were all in agreement with Hardy-Weinberg equilibrium (HWE) in the control group.

2.3. DNA extraction and genotyping

Genomic DNA was extracted from peripheral whole blood samples using the QIAamp Blood DNA Mini Kit as described by the manufacturer (Qiagen, Hilden, Germany). The SNP genotyping was performed with the Sequenom MassARRAYiPLEX Gold platform (Sequenom, Life Technologies, Shanghai) according to the manufacturer's instructions. The polymerase chain reaction (PCR) and extension primers were designed using MassARRAY Assay Design 3.1 software (see Table S2).

2.4. Haplotype analysis

Haplotype blocks in MyD88, IRAK4 and TRAF6 genes were selected with Haploview software version 4.2 (Broad Institute) by considering LD blocks. Haplotype frequencies and effects were examined using the SHEsis, a powerful software platform for analyses of haplotype construction (Shi and He, 2005). The detailed method was described by Li Z et al. (Li et al., 2009b). The global

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