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Short Communication

Association of P213S polymorphism of the L-selectin gene with type 2 diabetes and insulin resistance in Chinese population

Jing Liu [a,*,1], Ju-xiang Liu [a,c,1], San-ni Xu [b], Jin-xing Quan [a], Li-min Tian [a], Qian Guo [a], Jia Liu [a], Yun-fang Wang [c], Zhi-yong Shi [c]

[a] Department of Endocrinology, Gansu Provincial People's Hospital, 204 West Donggang Road, Lanzhou City 730000, Gansu Province, China
[b] The First People's Hospital of Tianshui City, Tianshui City 741000, Gansu Province, China
[c] The First Clinical College of Lanzhou University, Lanzhou City 730000, Gansu Province, China

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ABSTRACT

Aims: L-selectin belongs to selectin family of adhesion molecule and participates in the generation and development of type 2 diabetes (T2D). In this study, we evaluated the relationship between the P213S polymorphism of L-selectin gene and T2D and insulin resistance in the Chinese population.
Methods: We genotyped P213S polymorphism in 801 patients with T2D and 834 healthy controls in the Chinese population using polymerase chain reaction–ligase detection reaction (PCR–LDR) technique. Plasma glucose, insulin, lipid, blood urea nitrogen, creatinine and uric acid levels were measured by biochemical technique.
Results: The frequency of 213PP genotype and P allele of the L-selectin gene in patients with T2D was significantly higher than that in controls ($P=0.007$; $P=0.019$, respectively). The relative risk of allele P suffered from T2D was 1.191 times higher than that of allele S. Moreover, the levels of FPG and HOMA-IR of PP and PS genotype carriers were significantly higher than those of SS genotype carriers in the T2D group ($P<0.05$).
Conclusion: These findings indicated that the P213S polymorphism of L-selectin gene may contribute to susceptibility to T2D and insulin resistance in the Chinese population, and P allele appears to be a risk factor for T2D.

1. Introduction

T2D is an endocrine homeostasis disorder in sign of hyperglycosemia. It is characterized by peripheral insulin resistance, dyslipidemia, increased hepatic gluconeogenesis, loss of glucose induced insulin secretion and obesity (Weyer et al., 1999). L-selectin belongs to adhesion molecule family (Kadono et al., 2002), and it plays an important role in participating in the pathogenesis and development of T2D (Sugimoto et al., 1997).

Adhesion molecules are glycoprotein materials, located in the surface of the cells. They produce a marked effect depending on ligand-acceptor form, mediate the adhesion among the formed elements in the blood vessel (such as platelet, leucocyte) and vascular endothelium, and play an important role in the pathophysiology process about organism embryonic development, on coma transfer, inflammation, immune response, thrombosis and so on (Stollberger and Finsterer, 2002). Selectin family belongs to adhesion molecules, including L-selectin, E-selectin and P-selectin (Atalar et al., 2001). They have similar molecular structure, containing an N-terminal C-type lectin domain, a single EGF domain, a variable number of short consensus repeat (SCR) domains, a transmembrane region and a cytoplasmic tail (Bevilacqua and Nelson, 1993; Kansas, 1992). L-selectin resides in all neutrophil and mononuclear cell, most of the T lymphocyte, B lymphocyte, eosinophile granulocyte and some natural killer cell, however majority of them reside in neutrophil (Kansas, 1996). Its ligand distributes endothelial cell and some leucocyte, and most of the ligand belong to glucoprotein (Vestweber and Blanks, 1999). L-selectin not only mediates neutrophil and lymphocyte adhering activated endothelial cell, but also is regarded as a specific homing acceptor which mediates some lymphocyte subgroup homing periphery lymphaden (Carlos and Harlan, 1994). L-selectin will shed from cell surface when it suffers

Abbreviations: T2D, type 2 diabetes; PCR–LDR, polymerase chain reaction–ligase detection reaction; SCR, short consensus repeat; CAD, coronary artery disease; SMI, silent myocardial ischemia; GD, Graves' disease; T1D, Type 1 diabetes; WHO, World Health Organization; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; BMI, body mass index; WHR, waist hip ratio; FPG, fasting plasma glucose; FINS, fasting insulin; HOMA-IRI, insulin resistance index; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratios; CI, confidence intervals; SBP, systolic blood pressure; DBP, diastolic blood pressure; BUN, blood urea nitrogen; CR, creatinine; UA, uric acid.

* Corresponding author at: Department of Endocrinology, Gansu Provincial People's Hospital, 204 West Donggang Road, Lanzhou City 730000, Gansu Province, China. Tel./fax: +86 931 8281676.
E-mail address: liujingwelcome@126.com (J. Liu).
[1] Contributed equally to this work.

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stimulus of some cytokine, so it is easy to detect soluble L-selectin in blood serum (Bennett et al., 1996; Feehan et al., 1996; Preece et al., 1996; Stoddart et al., 1996). Although it is indefinite about the shedding mechanism, it has been certified that the cause of L-selectin shedding is protease splitting its membrane-proximal region (Bennett et al., 1996; Feehan et al., 1996; Preece et al., 1996; Stoddart et al., 1996).

In T2D patients with symptomatic coronary artery disease (CAD) or silent myocardial ischemia (SMI) associated with coronary stenoses, sL-selectin is significantly decreased (Kretowski and Kinalska, 2000). A marked fall in sL-selectin might constitute a marker for silent CAD in T2D patients (Albertini et al., 1999). Earlier studies had shown that polymorphonuclear neutrophils play an important role in the pathogenesis of diabetic vascular complications. Stimulation of these cells is associated with the desquamation of L-selectin (Karadayi et al., 2003). These results suggest that there is a strong relationship between sL-selectin and diabetic retinopathy (Karadayi et al., 2003). The strong correlation between sL-selectin and HbA1c levels supports the concept that the sL-selectin level is increased with poor glycemic control, which may affect endothelial cell activity and cause subsequent microvascular complications (Karadayi et al., 2003).

Insulin resistance and progressive β-cell failure are key factors in the pathogenesis of T2D (Liu et al., 2008). L-selectin combining with its monoclone antibody and ligand can cause cellular morphologic change, cellular framework rearrangement, oxygen respiration explosion, increasing IL-8 and TNF-α gene expression (Chen et al., 2006; Crockett-Torabi and Ward, 1996). As we know, TNF-α is one of the main factors that induces insulin resistance, so L-selectin may participate in the development of the insulin resistance. Thus, L-selectin plays an important role in the pathogenesis of the T2D (Kadono et al., 2002). The interaction of the LFA-1 located in lymphocyte and ICAM-1 located in β-cell may transmit synergetic stimulus signal, thereby activate T lymphocyte and mediate cytotoxicity T lymphocyte killing β-cell immediately (Green et al., 2006). Abnormal levels of some serum soluble adhesion molecules (ICAM-1, VICAM-1, E-selectin, P-selectin and L-selectin) have been detected in patients with T2D (Bannan et al., 1998; Kamiuchi et al., 2002). There is some evidence that serum levels of soluble adhesion molecules depend on glycemic control, disturbances of lipid metabolism, obesity and insulin resistance (Sokup, 2005). There are a few of clinical data till now, which have been suggesting the significant role of adhesion molecules in pathogenesis of microangiopathy and macroangiopathy in T2D (Kamiuchi et al., 2002). It is possible that in some cases measurement of serum adhesion molecules may be useful for predicting, early diagnosing prophylactic and monitoring of treatment of type 2 diabetic complications (Sokup, 2005). The study showed that they can modify serum levels of soluble adhesion molecules by nonpharmacologic and pharmacologic treatment (Sokup, 2005).

The chromosomal location of L-selectin is in 1q23-q25 (Iida and Nakamura, 2005), and the total length of the L-selectin gene, which is composed of nine exons and eight introns, is about 30 kb (Collins et al., 1991). Kretowski et al. (Kretowski and Kinalska, 2000) found that L-selectin gene T668C polymorphism was associated with T1D. L-selectin gene polymorphisms were associated with Graves' disease (GD) susceptibility in Chinese patients (Chen et al., 2007). However, there was no report about the relationship between the P213S polymorphism of L-selectin gene and T2D for a Chinese people. Thus, the aim of this study was to investigate the association of P213S polymorphism of L-selectin gene with T2D in a population from Northwest China using PCR–LDR. We also tested for association with the levels of insulin resistance.

2. Materials and methods

2.1. Study subjects

This study was approved by the Ethics Committee of Lanzhou University. After providing written informed consent, eight hundred and one T2D patients(case group)and 834 healthy subjects (control group) were recruited from the First Hospital and the Second Hospital of Lanzhou University, as well as the People's Hospital of Gansu Province in 2007–2010. All study subjects were asked not to use any alcohol-containing drinks or tobacco products over 24 h preceding the study. They were Chinese people residing in Gansu Province and had no family history of diabetes and no history of significant concomitant diseases. Exclusion criteria were ethanol or/and cigarette abuse, acute or chronic hepatopathy and nephropathy, heart diseases, Type 1 diabetes(T1D), abnormal laboratory test results and abnormal clinical signs or symptoms. All T2D patients (447 males and 354 females aged 56.45 ± 11.68 years), who were newly-diagnosed and unrelated diabetics, were diagnosed according to the criteria recommended by the World Health Organization (WHO) in 1999 (World Health Organization, 1999). And these patients had not received any treatment of hypoglycemic medicine such as insulin injection therapy and oral hypoglycemic agents. The non-diabetic subjects consisted of 834 health volunteers (471 males and 363 females aged 54.77 ± 11.62 years) were ethnically unrelated healthy subjects recruited from individuals matched by age and sex during the same period in the three hospitals. They had either a normal 75 g oral glucose tolerance test or a fasting glucose level below 6.0 mmol/l (108 mg/dl).

2.2. Laboratory measurements

Venous blood sample of 5 ml was drawn from all subjects into tubes containing ethylenediamine tetraacetic acid after an overnight fast. Fasting plasma glucose, fasting insulin, blood fat, blood urea nitrogen, creatinine and uric acid were measured by biochemical technique in a Clinical Laboratory in Gansu Provincial People's Hospital. Blood samples were collected in the morning after the participants had been fasting for at least 8 h. The levels of fasting plasma glucose were measured by a hexokinase glucose dehydrogenase method (Sysmex, Kobe, Japan),serum insulin levels were determined by means of electrochemiluminescence immunoassay, ECLIA (Elecsys 2010, Hitachi, Ltd., Tokyo, Japan; Roche Diagnostics, Rotkreuz, Switzerland). Plasma total cholesterol (TC), triglyceride (TG) and high-and low-density lipoprotein-cholesterol (HDL-C and LDL-C) were measured by an automated chemistry analyzer (Olympus AU 5400, Japan) with an enzymatic kit (Roche Diagnostics GmbH, Basel, Switzerland).

Anthropometric measurements from cases and control subjects were done in our ward and in Medical Examination Center, respectively. They were measured by standard technique. Body mass index (BMI) was calculated as weight in kilograms divided by square of height expressed in meters. Waist hip ratio (WHR) was calculated as waist circumference divided by hip circumference. The homeostasis model assessment of insulin resistance (HOMA-IR) was performed using the following formula: fasting insulin (IU/ml) × fasting glucose (mmol/l)/22.5.

2.3. PCR amplification

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood from study subjects according to a standard phenol–chloroform extraction method. Amplification of L-selectin gene fragment (137 bp) was performed with a pair of primers: upstream primer 5′-TTCAGTGT GAGCCTTTGGAG-3′ and downstream primer 5′- GGTGGTTTCTTCAA TCCCAGT-3′ (designed using Oligo6. Primer analysis software). The reaction mixture (20 μl) contained 2 μl of 1 × PCR Buffer, 0.3 μl of Taq DNA polymerase,4 μl of Q-solution (QIAGEN), 2 μl of 100 × deoxynucleoside triphosphates (Promega, 2 mM/each), 7.5 μl of sterile H2O and 2 μl of each primer (2pM/l).

The PCR conditions were as follows. Initial denaturation was performed at 95 °C for 15 min, and it was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 1 min and extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. To