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Association between the leptin receptor gene polymorphism and lipoprotein profile in Chinese type 2 diabetes

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Summary

Objective: Leptin has been shown to have a major influence on plasma lipid and lipoprotein metabolism through leptin receptors (LEPR). Genetic studies have been implicated common variants in the human LEPR gene were associated with hyperinsulinemia, type 2 diabetes and leptin levels. In this study, we examined the distribution of the LEPR gene G3057A polymorphism and the association between plasma leptin levels and lipoprotein profile in Chinese type 2 diabetes.
Methods: 186 unrelated patients with type 2 diabetes and 150 normal glucose tolerances (NGT) controls were recruited. Hemi-nested PCR-RFLP and PCR direct sequence analysis were used to analysis the LEPR G3057A genotype. Plasma leptin levels were measured using radioimmunoassay kit. Plasma lipid levels triglycerides (TG), total cholesterol (CH), high density lipoprotein lipoprotein cholesterol (HDL-C), low density lipoprotein lipoprotein cholesterol (LDL-C), apolipoprotein AI and apolipoprotein B were measured routinely after overnight fasting.
Results: The frequency of the mutant A allele was 57% in diabetic patients versus 56% in normal glucose tolerances controls. The distribution of the three genotypes in control group was as follows: GG genotype, 36.0%; GA genotype, 15.3%; and AA genotype, 48.7%. This genotype distribution did not differ between control subjects and type 2 diabetic patients in which 33.3% were GG, 18.3% were GA and 48.4% were AA ($\chi 2 = 1.17$, $P > 0.05$). Plasma leptin levels were significantly higher in the diabetic patients (35.72 ± 2.85) than those in NGT subjects (8.67 ± 0.35) , but there was no statistical difference between LEPR genotypes and plasma leptin levels in the diabetic patients or in NGT control subjects. However in type 2 diabetic groups, AA genotype at nucleotide 3057 had higher levels of TG and LDL-C, and lower levels of HDL-C ($P < 0.01$).

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Conclusion: The LEPR G3057A *mutation* was common in the Chinese population, this polymorphism was not associated with the glucose metabolic parameters and plasma leptin levels in Chinese subjects, but positively associated with plasma TG and LDL-C levels, negatively associated with plasma HDL-C levels in Chinese diabetic patients. It may contribute to the onset of type 2 diabetes by regulating lipid metabolism and affecting the distribution of local body mass.

Introduction

Adipose tissue plays a crucial role in the regulation of energy homeostasis, insulin sensitivity, and lipid/carbohydrate metabolism. These actions are mediated by the actions of a number of hormones, there are three main adipocyte hormones produced in adipocytes, they are leptin, acylation stimulating protein (ASP), and adiponectin. Leptin has been shown to stimulate glucose uptake and fatty acid oxidation in skeletal muscle [1,2], to prevent lipid accumulation in nonadipose tissues such as skeletal muscle, liver, and pancreatic-cells [3], it has been shown to have a major influence on lipid and lipoprotein metabolism through leptin receptors (LEPR).

Mutations in the leptin gene resulting in leptin deficiency cause obesity, insulin resistance, and diabetes in animals [4]. Mutations resulting in a deficient leptin receptor cause obesity and diabetes in animals [5,6] and obesity in humans [7]. Common variants in the human LEPR gene have been associated with hyperinsulinemia [8,9], type 2 diabetes [8], obesity, and leptin levels [10—13].

The regulatory role of leptin on lipid metabolism was known, but the relationship between leptin, LEPR gene polymorphism and plasma lipid profile varies with different genetic population. A study in Pakistan diabetic postmenopausal women discovered that non-significant correlation existed between leptin and lipid levels in normal and diabetic patients. Significant negative correlation existed between leptin and HDL ($r = -0.30$) in diabetic patients [14]. A study in Thai obese and overweight subjects discovered that serum leptin level was positively associated with HDL-C and negatively with LDL-C/HDL-C [15]. Another study in Nauruan males reported that Ob-R Pro1019Pro (G3057A) polymorphism was associated with serum insulin levels and not associated with serum lipid profile levels [16]. These conflicting results may be due in part to differences in the genetic backgrounds of the studied populations. These results prompted us to explore the association between LEPR gene G3057A polymorphism in exon 20, plasma leptin levels and lipoprotein profile in Chinese population.

Materials and methods

Subjects

About 186 unrelated Chinese patients with type 2 diabetes mellitus and 150 controls were recruited. Diabetes mellitus was diagnosed according to the criteria published by the World Health Organization in 1999. They had no evidence of current acute illness including clinically significant infectious disease. The duration of diabetes was 9.6 ± 2.7 year (range 0—32 year). An age matched normal glucose tolerance (NGT) controls ($n = 150$, mean age 59.3 ± 6.82 years, 77 males, 73 females) were recruited at the routine healthy examination.

Plasma parameters determination

Fasting blood samples were obtained for measurement of glucose, HbA1c, and lipid levels. Routine clinical biochemical analyses were performed in the hospital laboratory. Plasma glucose was measured with glucose oxidase method. Plasma triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), apolipoprotein AI, apolipoprotein B were measured with oxides method by automatic biochemistry instrument, Low-density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald's formula [17]. Plasma insulin was measured using a two-site immunoradiometric assay. Serum leptin levels were determined by a kit obtained from DRG Instruments (Germany) and the samples were analyzed with ELISA [18].

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes by the phenol chloroform method. Heminest PCR was performed on the genomic DNA samples with a Gene Amp PCR kit (Promega) and primers as previously reported [19]. The primers were as follows: P_0, 5′-ACTGTGGTCTCTCTACTTTC-3′; P_1, 5′-TTTGATAGAGAAGC ACTTGG-3; P_2, 5′-CCATGAGCTATTAGAGAAAGAATCCGTCAA-3′. The PCR mixture contained 1 μmol/L of each primer (P_0/P_2), 2U of Taq polymerase, 25 mmol/L $MgCl_2$, 0.2 mol/L of each