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Relationship between plasma HDL subclasses distribution and lipoprotein lipase gene *Hind*III polymorphism in hyperlipidemia

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Abstract

Background: Different high-density lipoprotein (HDL) subclasses have distinct but interrelated metabolic functions. HDL directly influences the atherogenic process, and changes in HDL subclasses distribution may be related to the incidence and prevalence of atherosclerosis. Lipoprotein lipase (LPL) is an important enzyme for hydrolysis of triglyceride-rich lipoproteins, and its activity is positively correlated with the plasma HDL cholesterol level. LPL gene *Hind*III polymorphism has been found associated with variations in lipid levels, but the impact on HDL subclasses distribution is less clearly established.

Methods: The relative apolipoprotein (apo) A-I contents (% apoA-I) of plasma HDL subclasses were determined by two-dimensional gel electrophoresis coupled with immunodetection and LPL gene *Hind*III polymorphism was assayed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 173 hyperlipidemic and 155 normolipidemic subjects.

Results: The frequencies of 495TT genotype and allele T were the highest both in the hyperlipidemic and control groups. Compared with the control group, the frequency of 495TT genotype was higher, while the frequencies of 495TG and 495GG genotypes were significantly lower ($P<0.05$) in the hyperlipidemic group. Two-dimensional gel electrophoresis and immunodetection showed that HDL subclasses distribution was altered in hyperlipidemia, and had a general shift toward smaller size. Compared with the control group, the hyperlipidemic group had significantly higher relative apoA-I contents of preβ_1-HDL, preβ_2-HDL, HDL_{3b} and HDL_{3a} ($P<0.05$) and lower HDL_{2a} and HDL_{2b} levels ($P<0.001$). In the hyperlipidemic group, allele T carriers' frequency was higher than that in the control group ($P<0.05$), and the genotype of 495TT showed higher levels of plasma TG, apoB100, TG/HDL-C ratio, relative apoA-I contents of preβ_1-HDL, HDL_{3b} and lower HDL_{2a}, HDL_{2b} compared with that of the 495GG genotype subgroup ($P<0.05$). In the control group, the genotype of 495TT had higher plasma TG, HDL_{3c} and lower HDL_{2a} compared with that of 495GG subgroup ($P<0.05$).

Conclusions: The 495TT genotype of LPL gene *Hind*III polymorphism was associated with changes of HDL subclasses distribution in Chinese population with hyperlipidemia. The particle size of HDL shifted toward smaller, which, in turn, indicated that RCT might be weakened and HDL maturation might be abnormal in hyperlipidemic subjects with 495TT genotype.

Keywords: Hyperlipidemia; Lipoprotein lipase; HDL subclasses; Gene polymorphism; Two-dimensional gel electrophoresis-immunodetection

1. Introduction

Numerous clinical and epidemiological studies have demonstrated that the level of plasma high-density lipoprotein cholesterol (HDL-C) is inversely correlated with

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coronary heart disease (CHD). High-density lipoprotein (HDL) particles have multiple biologic effects that could contribute to their anti-atherogenic function through anti-inflammatory, anti-oxidative, and profibrinolytic effects. Importantly, they can also promote the reverse cholesterol transport (RCT) [1–7].

HDL is a heterogeneous class of lipoprotein particles with subclasses that differ in apolipoprotein and lipid composition, size, density, and charge. The different subclasses appear to have varied physiological functions [8]. Our laboratory compares plasma HDL subclasses by two-dimensional gel electrophoresis, immunoblotting, and subsequent quantitative imaging. HDL can be separated into two parts, i.e., pre-β and α-HDL. The pre-β part can be further distinguished by subsequent polyacrylamide gradient gel electrophoresis into pre-β_1, pre-β_2HDL, and α-HDL can be separated into five distinct subclasses ($HDL_{3c,\ 3b,\ 3a,\ 2a,\ 2b}$) according to their increasing particle sizes [9–11]. Nascent discoidal preβ-HDL is converted to mature spherical HDL_2 via HDL_3 [12,13]. Plasma factor as well as several enzymes and protein factors, such as lecithin cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL) and hepatic lipase (HL) together with cholesteryl ester transfer protein (CETP) and phospholipids transfer protein (PLTP) are involved in this process. Due to the importance of RCT in anti-antherosclerosis, changes in HDL and HDL subclasses may directly influence the antherogenic process and could therefore correlate with incidence and prevalence of CHD [5]. Many retrospective and prospective studies have identified that small-size HDL subclasses contribute to increased risk of CHD while large-size HDL subclasses are associated with decreased risk [10,14–17].

LPL cleanses triglyceride-rich lipoproteins from circulation through its role in hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL). LPL also promotes the exchange of lipids between VLDL and HDL. Because of its key role in lipid metabolism, LPL is likely to have important influence in the development of atherosclerosis and CHD [18–20].

The LPL gene is located on the short arm of chromosome 8, spanning about 35 kb and containing 10 exons, and several restriction fragment length polymorphisms (RFLP) have been identified. Defects in LPL gene can lead to lipid disorder and possibly increased risk of atherosclerosis. *Hind*III polymorphism, a thymine (T) to a guanine (G) base transition at position +495 in intron 8, which abolishes a *Hind*III restriction enzyme recognition site, is one of the most common polymorphisms in the LPL gene [21–27].

HDL subclasses distribution and LPL variants probably modulate the risk for dyslipidemia and increase the risk of CHD, but the relationship between LPL gene *Hind*III polymorphism and HDL subclasses distribution have been investigated in population with hyperlipidemia and a group of normolipidemic subjects.

2. Subjects and methods

2.1. Subjects

The subjects in this study consisted of 328 (210 males and 118 females) were current or retired staff. One hundred eighty subjects came from West China University of Medical Sciences, Sichuan University and Sichuan Normal University, in Chengdu, Sichuan Province, and the others (148 subjects) were from Nanhua University, in Hengyang, Hunan Province, P.R. China. According to the third Report of NCEP (ATP-III) guideline [28], the 328 individuals were divided into 2 groups. The control group included 155 normolipidemic subjects aged 33–78 y (mean 53.6±11.3 y), who were apparently healthy, with plasma TG<2.26 mmol/l and total cholesterol (TC)<6.21 mmol/l, and without smoking or using lipid-lowering drugs. The hyperlipidemia group included 173 hyperlipidemic subjects aged 34–77 years (mean 54.7± 11.8 y), with plasma TG≥2.26 mmol/l and/or TC≥6.21 mmol/l, and who had not been treated with lipid-lowering drugs in the previous one month, and all secondary causes of hyperlipidemia were excluded by appropriate tests.

2.2. Specimens

Whole blood specimens were drawn after a 12-h overnight fast into EDTA-containing tubes. Plasma was separated within 1–2 h, stored at 4 °C and used within 24 h for lipid and apolipoprotein analyses. An aliquot of plasma was stored at −70 °C for the determination of HDL subclasses and 2 ml whole blood for extracting genomic DNA to investigate LPL *Hind*III polymorphism.

2.3. Plasma lipid and apolipoprotein analyses

Plasma TG, TC and HDL-C were measured by standard techniques. TC and TG were determined with enzymatic kits (Beijng Zhongsheng Biotechnological Corporation, Beijing). HDL-C was determined after precipitation of the apolipoprotein (apo) B-containing lipoproteins by phosphotungstate/magnesium chloride [29]. When TG<4.52 mmol/l, Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedwald formula [30]. When TG≥4.52 mmol/l, LDL-C was determined with enzymatic kits (Beijing Zhongsheng). Plasma apoA-I, B100, C-II, C-III and E were determined with radial immunodiffusion methods [31] using kits developed by Apolipoprotein Research Laboratory, West China Medical Center, Sichuan University.