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Short Communication

Chitinase 3-like 1 gene-329*G*/*A* polymorphism, plasma concentration and risk of coronary heart disease in a Chinese population

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ABSTRACT

Background: The chitinase-like 1 protein, YKL-40, is involved in inflammation and tissue remodeling. Patients with coronary heart disease (CHD) and acute myocardial infarction have elevated levels of serum YKL-40. The goal of the present study was to investigate whether the chitinase-like 1 gene-329*G*/*A* variant (rs10399931) confers susceptibility to CHD, and whether it is associated with the clinical phenotype and severity of disease.

Methods: We performed a case-control study of 410 unrelated CHD patients (coronary stenosis ≥50% or documented myocardial infarction) and 442 controls from China. A ligase detection reaction was used to determine a single-nucleotide polymorphism in rs10399931. The genotypic and allelic associations of this single-nucleotide polymorphism with CHD, phenotypes and severity were also evaluated. Plasma levels of YKL-40 were measured using ELISA assays.

Results: Three genotypes, *CC*, *CT*, and *TT*, existed in rs10399931 and there were no significant differences found in either the genotypic or allelic frequencies between the CHD cases and controls. Patients with CHD had higher YKL-40 levels compared to controls and those with acute myocardial infarction had the highest levels of YKL-40 compared to patients with either stable or unstable angina pectoris (all $p<0.01$). Rs10399931 affected neither the main anthropometric or metabolic characteristics, nor did there exists any association between rs10399931 and the severity of coronary lesions assessed by Gensini scores (all $p>0.05$).

Conclusions: Our results do not support that rs10399931 is associated with clinical phenotypes of CHD and the extent of coronary lesions; however, YKL-40 levels are higher in CHD patients and associated with its clinical phenotypes.

1. Introduction

Coronary heart disease (CHD) is a common, disabling disorder and the leading cause of death in the world. Recently, studies have shown a greater understanding of the genetic background of folate metabolism in animal models (Wakefield et al., 2010), CHD (Samani et al., 2007) and other clinical disorders (Plummer et al., 2011). Therefore, an interest in determining measurable humoral bio and genetic markers to improve global cardiovascular risk stratification and guide therapeutic decision-making has emerged.

Atherosclerosis is a central feature of CHD and is a known inflammatory disease involving the activation of endothelial cells, infiltration of monocytes into the vessel wall and differentiation of macrophages into lipid-laden foam cells (Libby, 2002). However, the molecular mechanisms of macrophage behavior in atherogenesis are not entirely understood.

The chitinase 3-like 1 gene (CHI3L1 [OMIM 601525]) is located on chromosome 1q32.1 (Johansen et al., 2006) and encodes the CHI3L1 protein, also known as YKL-40, or the human cartilage glycoprotein 39 (HC gp-39) (Hakala et al., 1993). YKL-40 was recently found to be pro-inflammatory and is released by activated human macrophages (Boot et al., 1999), vascular smooth muscle cells (VSMCs) (Shackelton et al., 1995), and neutrophils (Volck et al., 1998). It also plays a vital role in the pathogenesis of endothelial dysfunction, atherosclerosis, and abnormal angiogenesis (Rathcke and Vestergaard, 2006). Recently, YKL-40 has been a promising biomarker for acute

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; CAG, coronary angiography; CHD, coronary heart disease; CVD, cardiovascular disease; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAP, stable angina pectoris; SNP, single nucleotide polymorphisms; TC, total cholesterol; T2DM, type 2 diabetes mellitus; TG, triglyceride; UAP, ceunstable angina pectoris; VSMCs, vascular smooth muscle cells.

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and chronic inflammation (including systemic low-grade inflammation) and tissue remodeling (Johansen, 2006; Libby, 2002; Rathcke and Vestergaard, 2006; Shackelton et al., 1995).

YKL-40 is a product of activated macrophages. Either the concentration of YKL-40, or the chitotriosidase activity, may be useful in the identification of atherosclerosis and its stability. An increased expression of YKL-40 mRNA levels has been shown in the carotid plaques of patients with ischemic symptoms (Michelsen et al., 2010). There are also elevated serum YKL-40 levels and chitotriosidase activity in patients with carotid atherosclerosis and CHD (Artieda et al., 2003; Michelsen et al., 2010). The serum levels of YKL-40 and chitotriosidase activity were associated with the severity of atherosclerotic lesions defined by the number of stenosed vessels (Artieda et al., 2003; Kucur et al., 2007). Furthermore, increased serum YKL-40 levels are independently associated with the progression of coronary lesions in patients with CHD (Zheng et al., 2010). Another study reported that serum YKL-40 levels are higher in patients with acute myocardial infarction (AMI) than in patients with either stable CHD or control subjects (Nøjgaard et al., 2008).

Data are inconsistent when considering the association between CHI3L1-329*G*/*A* polymorphism (rs10399931) and either serum YKL-40 levels or chitotriosidase activity. In one study, serum YKL-40 levels in controls were dependent on the genetic variant at rs10399931 (Kruit et al., 2007); however, this association did not exist in patients with sarcoidosis. YKL-40 levels were elevated in patients with type 1 (Rathcke et al., 2009a, 2009b) and type 2 diabetes mellitus (T2DM) (Nielsen et al., 2008) and were also associated with vascular complications (Rathcke et al., 2009a, 2009b). Depending on the severity of the atherosclerotic lesions, serum chitotriosidase activity was higher in patients containing either atherothrombotic stroke or ischemic heart disease than in controls. These observed differences in activity were not attributed to a distinct genotypic or allelic distribution (Artieda et al., 2003).

These studies have failed to provide any genetic basis for the association between the *CHI3L1* gene variant and CHD. In addition, little is known regarding the relationship between the presence of rs10399931, clinical phenotypes and the extent of CHD in Chinese populations. Therefore, the aim of the present study was to investigate the role of single nucleotide polymorphisms (SNP) within the genetics of CHD in a Chinese population, and to assess whether the presence of SNP is correlated with CHD and the severity of coronary lesions assessed by Gensini scores.

2. Methods

2.1. Study subjects

Any of the 852 patients undergoing elective coronary angiography (CAG) for either chest discomfort or suspected CHD were included in the study (data collected from June 2004 to June 2011). The total cases included 410 subjects (men aged 31–79 years and women aged 37–81 years) with documented CHD. According to the World Health Organization criteria, CHD was defined as significant coronary stenosis (≥50%) in at least either one of the three main coronary arteries or their major branches (branch diameter ≥2 mm). It was assessed using CAG or if a subject showed a previous diagnosis of a myocardial infarction (MI). All patients with previous MI had confirmatory CAG findings. CHD cases were divided into three subgroups, including: stable angina pectoris (SAP), unstable angina pectoris (UAP), and AMI. The definitions of SAP, UAP, and AMI were in accordance with current guidelines. We also included the 442 sex- and age-matched controls that were without detectable coronary stenosis and assessed via CAG. All patients with congenital heart disease, either Prinzmetal type angina or variant angina, syndrome X, multiple aorto-arteritis, severe liver or kidney disease, or contraindications for heparin usage were excluded from the present study. All subjects who participated in the study provided a written informed consent, and the study protocol was approved by the Medical Ethics Committee of the Affiliated Zhongda Hospital of Southeast University.

2.2. Coronary angiography

All patients underwent elective CAG according to the Judkins technique, and images were recorded offline for later analyses. Gensini scores were calculated to reflect the extent of coronary lesions (Gensini, 1983). All patients were recruited from the same geographical area with similar socioeconomic and ethnic backgrounds.

2.3. Determination of risk factors

Data were collected from each subject at the time of enrollment, including a complete survey of the following CHD risk factors: history of hypertension, T2DM, family history of cardiovascular disease (CVD), and smoking status. Anthropometric measurements were performed according to standard protocols. Hypertension was diagnosed as either systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg (average of three repeated measurements performed by the same doctor) or from either previous diagnosis or prescription for antihypertensive medications. Subjects with a history of T2DM, those receiving anti-diabetic medications, and those with confirmed fasting blood sugar >126 mg/dL (7.0 mmol/L) were considered as having T2DM. A positive family history of CVD was documented when a physician confirmed diagnoses in a first-degree relative. Subjects who smoked at least one cigarette per day at the time of enrollment were considered active smokers, as were those who had smoked in the month prior to the study. Blood was collected and total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined using standard biochemical methods including a chemistry analyzer (Beckman Coulter Synchron clinical system LX20). Dyslipidemia was defined by the presence of at least one of the following criteria: fasting TC ≥5.18 mmol/L (200 mg/dL), TG ≥1.70 mmol/L (150 mg/dL), LDL-C ≥3.37 mmol/L (130 mg/dL), and HDL-C <1.04 mmol/L (40 mg/dL). Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2).

2.4. Genotyping and YKL-40 concentration measurement

Extraction of DNA from peripheral whole blood was performed following the manufacturers' instructions using a commercially available kit [Axygene Biotechnology Limited (Hangzhou, China)]. The characterization of rs10399931 was performed using a ligase detection reaction (Xiao et al., 2006) with TaqMan genotyping assays on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA). Meanwhile, repeat genotyping was performed on random duplicate samples and sequencing techniques were used to ensure quality control. The plasma levels of YKL-40 from 270 individuals (81 in controls and 189 in the CHD group) were measured using enzyme-linked immunosorbent assays (R&D kit, USA) with a sensitivity of 0.01 μg/L.

2.5. Statistical analyses

Statistical analyses were conducted using SPSS 15.0 for Windows (SPSS, Inc., Chicago, Illinois). Data were expressed as an absolute number (percentage) or mean ± standard deviation. A Student's *t* test and chi-square test were used to analyze differences between the two study groups. Allele and genotype frequencies between CHD cases and controls were compared with values predicted by the Hardy–Weinberg equilibrium using the chi-square test. Analyses of continuous data and categorical variables among three different groups according to the genotypes in rs10399931 were determined