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Human C-reactive protein gene polymorphism and metabolic syndrome are associated with premature coronary artery disease

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ABSTRACT

The aim of this study was to investigate the association between C-reactive protein (CRP) gene polymorphism and metabolic syndrome (MetS) with premature coronary artery disease (PCAD). 116 patients with PCAD (58 with MetS and 58 without MetS) and 119 controls were included in the study. CRP gene +1059 G>C polymorphism was analyzed by polymerase chain reaction. Serum hs-CRP was measured using high-sensitivity enzyme-linked immunosorbent assay. Carriers of C allele of the CRP +1059 G>C polymorphism had 3.37 fold increased risk to develop MetS in patients with PCAD. In addition CRP gene and hs-CRP levels were independent risk factors for PCAD and MetS. The present study provides new evidence that the presence of CRP +1059 G>C polymorphism and hs-CRP levels are independent determinants of PCAD and MetS in Egyptians. The results of our study suggest a synergistic effect of CRP C allele with classical risk factors such as hypertension, obesity, dyslipidemia and MetS.

1. Introduction

Inflammation plays an important role in the initiation and progression of atherosclerosis (Speidl et al., 2002). A strong association between high-sensitivity C-reactive protein (hs-CRP) and cardiovascular disease was established in previous studies in healthy subjects and in patients with coronary artery disease (CAD) (Ikonomova, 2004).

The realization that certain CRP genetic polymorphisms influence blood CRP levels could be of great clinical importance, because genetic predisposition to high baseline CRP might increase the risk of CAD in a significant proportion of people (Montecucco and Mach, 2008; Hingorani et al., 2006; Zebrack et al., 2002).

The human CRP gene is located on chromosome 1q21 to 1q23, spanning approximately 1.9 kb and containing two exons. Several single nucleotide polymorphisms (SNPs) in the CRP gene were reported to be associated with circulating CRP concentrations and/or risk of vascular disease (Carlson et al., 2005). The interplay between genetic and environmental factors is important in the phenotype development of complex traits as CAD. Elevated hs-CRP levels may be predictive of the development of the metabolic syndrome (Abdel-Aziz and Mohamed, 2013; Grassi et al., 2009; Haffner, 2006).

The presence of metabolic syndrome (MetS) imparts a high risk of development of early-onset premature CAD (PCAD) (Iribarren et al., 2006). In 2001, the National Cholesterol Educational Program Adult Treatment Panel III (NCEP-ATP-III, 2001) defined the metabolic syndrome as a clustering of 3 or more vascular risk factors, including abdominal obesity, high triglycerides, decrease in high-density lipoprotein cholesterol (HDLc) level, high blood pressure, and high fasting glucose. Because the prevalence of MetS is increasing among young adults due to adverse physical activity and dietary patterns (de Ferranti et al., 2004), it is important to quantify its relation with premature CAD.

Prior studies showed that CRP +1059 G>C polymorphism was associated with plasma concentrations of CRP (Zee and Ridker, 2002). This relationship was present in subjects with and without prevalent coronary disease in the Caucasian population (Suk et al., 2005). Another study showed that the CRP +1059 G>C polymorphism was neither associated with future myocardial infarction (MI) and stroke, nor with post-angioplasty restenosis (Zee et al., 2004). Therefore, its correlation with the pathogenesis of CAD remains unknown. Therefore, the aim of our study was to investigate the association between CRP gene polymorphism and metabolic syndrome with PCAD in the Egyptian population in Zagazig.

2. Methods

2.1. Study population

The total population of this study consisted of 235 Egyptian individuals divided into two groups: 116 unrelated individuals consisting of 90 males and 26 females (mean age 42.4 ± 7.3 years) with documented

Abbreviations: CAD, Coronary artery disease; CRP, C-reactive protein; ELISA, High-sensitivity enzyme-linked immunosorbent assay; MetS, Metabolic syndrome; HDLc, High-density lipoprotein cholesterol; hs-CRP, High-sensitivity C-reactive protein; LDLc, Low density lipoprotein cholesterol; PCAD, Premature coronary artery disease; SNPs, Single nucleotide polymorphisms; TC, Total cholesterol; TG, Triglycerides; TNF-α, Tumor necrotizing factor-α.

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CAD (PCAD group), age at the time of CAD diagnosis 45 years or less in men and 55 years or less in women, and 119 unrelated control subjects with mean age of 41.9 ± 6.4 years (63 males and 56 females). Patients with CAD were recruited from patients admitted to the Cardiology Section of the Zagazig University Hospital (Zagazig, Egypt). Documented CAD was diagnosed by the following criteria: stable CAD suggested by clinical evaluation and proved by coronary angiography (>50% reduction of coronary artery diameter in at least one of the major arteries) or the occurrence of myocardial infarction (MI) as defined by WHO criteria. The control subjects were randomly selected and were age matched to PCAD patients. They had no history of CAD, MI or stroke.

The presence of MetS was determined using the NCEP-ATP-III (2001) definition (waist circumference ≥102 cm in men or ≥88 cm in women, fasting triglycerides ≥150 mg/dl, HDL-cholesterol <40 mg/dl in men or <50 mg/dl in women, hypertension defined as blood pressure ≥130/85 mmHg or use of blood pressure medication, and impaired fasting glucose (IFG) ≥110 mg/dl).

The presence of CAD risk factors was determined using the criteria of the European society of cardiology: hypertensive condition was attributed when systolic blood pressure values were >139 mm Hg and/or diastolic blood pressure values >89 mm Hg in at least two separate measurements or when being medicated against hypertension; subjects were considered smokers when consuming more than five cigarettes per day or non-smokers when never smoked or had stopped smoking at least 1 year before sample collection; obesity was defined for waist-to-hip ratio of >0.90 in men or >0.85 in women; dyslipidemia was considered for serum values of total cholesterol (TC) ≥200 mg/dl, triglycerides (TG) ≥150 mg/dl, low density lipoprotein cholesterol (LDLc) ≥130 mg/dl and high density lipoprotein cholesterol (HDLc) ≤40 mg/dl in men and ≤50 mg/dl in women.

2.2. Analyses of lipid

Blood samples were drawn from all subjects after an overnight fast. Sera were separated immediately and stored at −20 °C. Total cholesterol and triglycerides were measured by routine enzymatic methods (spinreact). HDLc was determined after precipitation of the apoB-containing lipoproteins. LDLc was calculated using Friedewald et al.'s (1972) formula. Serum hs-CRP was measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) for CRP (Wu et al., 2002).

2.3. Isolation of DNA

Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany).

2.4. Genotyping of CRP gene +1059 (rs1800947) polymorphism

Genotyping of CRP gene +1059 polymorphism was performed by polymerase chain reaction (PCR) and further digestion by *Mae*III restriction enzyme, as previously described (Cao and Hegele, 2000). Briefly, PCR was performed by using the following primers: forward 5′-GATCTGTGTGATCTGAGAAACCTCT-3′ and reverse 5′-GAGGTACCAGAGA CAGAGACGTG-3′. 50 μl reaction mixtures containing 10 μl genomic DNA, 30 μl one step PCR mixture (1 unit Taq polymerase, 10 mM KCl, 10 mM$(NH_4)_2$ SO_4, 20 mM Tris HCl (pH 8.75), 0.1% Triton X-100, 0.1 mg/ml BSA and 200 μm dTNPs) and 2 μl of each primer (BioBasic Inc., Ontario, Canada) and 8 μl DdH_2O. Target DNA was amplified using 94 °C for 5 minutes, then 30 cycles of 94 °C for 30 seconds, 57 °C for 30 seconds and 72 °C for 30 seconds, followed by an extension step of 72 °C for 10 minutes. Two percent agarose gel was used for visualization. The PCR product size was 744 bp. Digestion of the less common C allele produces two smaller fragments, with sizes of 434 and 310 bp. Digestion of the more common G allele produces three fragments, with sizes of 310, 233, 201 bp.

2.5. Statistical analyses

The appropriate sample size and power of the study were determined using PAWE-3D (Laboratory of Statistical Genetics, New York) (Gordon et al., 2005). PAWE-3D calculations showed that the sample size, together with the specified study design, allele frequencies, prevalence of disease, and allowable error rates, can give as high as 90% power and can detect variant allele frequency of at least 0.05 and genotype relative risk of ≥1.8 at 80% power. Descriptive parameters are presented as mean ± SD and in percentages for variables with normal distribution. Comparisons between groups of means were performed using ANOVA. Chi-square test (χ^2) was used to compare categorical variables between the groups. Genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the χ^2 test. In addition, we calculated the odds ratios (ORs) and 95% confidence intervals (CIs) regarding the presence of PCAD with respect to the existence of polymorphism. To analyze possible positive or negative interactions between classical risk factors for CAD and genetic polymorphisms we used a 4 × 2 table approach to calculate ORs, respective 95% confidence intervals and *P* values, as well as synergy measures in additive (SI). It was assumed that unexposed individuals without the susceptibility genotype have a certain background risk for disease (OR_{00} is assumed to be 1); OR_{10} refers to the relative risk for disease among people without the susceptibility genotype for disease but exposed to the environmental risk factor relative to those with neither the susceptibility genotype nor exposure; OR_{01} refers to the relative risk among people with the susceptibility genotype who are not exposed to the risk factor relative to those with neither the susceptibility genotype nor exposure; OR_{11} is the ratio of disease risk among exposed people with susceptibility genotype to diseased risk among unexposed people without the susceptibility genotype. These ORs was then used in the calculation of synergy indexes: $SI = (OR_{11} - 1)/(OR_{10} + OR_{01} - 2)$ (Assman et al., 1996). Multiple regression analysis was performed. A difference was considered significant at $P < 0.05$. All data were evaluated using SPSS version 10.0 of the windows.

3. Results

3.1. Clinical data and biochemical characteristics of study subjects (Table 1)

The PCAD patients had a higher frequency of family history of CAD, diabetes, smoking and hypertension than the controls. Regarding lipid profile, the levels of total cholesterol, TG and LDLc were significantly increased in PCAD group compared to controls. Furthermore, levels of HDLc were significantly decreased in PCAD patients compared to control group. Levels of hs-CRP significantly increased in PCAD group compared to controls

3.2. Distribution of CRP genotype in PCAD patients and controls (Table 2)

The genotype frequencies of the CRP were in agreement with Hardy–Weinberg equilibrium in all groups. PCAD patients showed nonsignificant differences in genotype distributions compared with controls.

3.3. Distribution of CRP genotype in metabolic and non-metabolic PCAD patients (Table 3)

The metabolic PCAD group had significantly increased frequencies of the C allele compared to non metabolic PCAD ($P = 0.01$). PCAD subjects with CRP C allele were significantly more likely to develop metabolic syndrome (OR = 3.37, 95% CI = 1.29–8.82, $P = 0.01$).

3.4. Risk factors of PCAD (Table 4)

PCAD was tested for independence from other variables by multiple regression analysis. The model included sex, hypertension, diabetes,