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Genetic predisposition to left ventricular dysfunction: a multigenic and multi-analytical approach

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ABSTRACT

Background: Left ventricular dysfunction (LVD) is a complex, multifactorial condition, caused by mechanical, neurohormonal, and genetic factors. We have previously observed association of renin–angiotensin–aldosterone system (RAAS), matrix metalloproteinases (MMPs) and inflammatory pathway genes with LVD. Therefore the present study was undertaken to identify the combination of genetic variants and their possible interactions contributing towards genetic susceptibility to LVD in the background of coronary artery disease (CAD).
Methods and results: The study included 230 healthy controls and 510 consecutive patients with angiographically confirmed CAD. Among them, 162 with reduced left ventricle ejection fraction (LVEF ≤ 45%) were categorized as having LVD. We analyzed 11 polymorphisms of RAAS, MMPs and inflammatory pathways. Single locus analysis showed that *AT1* A1166C (p value < 0.001; OR = 3.67), *MMP9* R668Q (p value = 0.007; OR = 3.48) and *NFKB1-94* ATTG ins/del (p value = 0.013; OR = 2.01) polymorphisms were independently associated with LVD when compared with both non-LVD patients and healthy controls. High-order gene–gene interaction analysis, using classification and regression tree (CART) and multifactor dimensionality reduction (MDR) revealed that *AT1* A1166C and *NFKB1*-94 ATTG ins/del polymorphisms jointly increased the risk of LVD to great extent (p-value = 0.001; OR = 8.55) and best four-factor interaction model consisted of *AT1* A1166C, *MMP7* A-181G, *MMP9* R668Q and *NFKB1*-94 ATTG ins/del polymorphisms with testing accuracy of 0.566 and cross validation consistency (CVC) = 9/10 (permutation p < 0.001) showed increased risk for LVD respectively.
Conclusion: AT1 A1166C independently and in combination with *MMP9* R668Q and *NFKB1-94* ATTG ins/del polymorphisms plays important role in conferring genetic susceptibility to LVD in CAD patients.

1. Introduction

One of the major challenges in the human genetics is identifying sequence variations that lead to an increased risk of disease. In the case of rare single gene disorders the genotype–phenotype relationship is easily evident as the mutant genotype is explicitly responsible for the disease, but in case of complex, polygenic diseases, the relationship is extremely difficult to characterize, since the disease is likely to be the result of many genetic and environmental factors. In fact, epistasis, or gene–gene interaction is increasingly assumed to play a crucial role in genetic architecture of polygenic diseases (Gibbons et al., 2004).

Left ventricular dysfunction (LVD) is a complex, multifactorial condition, caused by several mechanical, neurohormonal, and genetic factors (Triposkiadis et al., 2009). It has been established that the extensive coronary artery disease (CAD) and/or myocardial damage is the leading cause of the LVD. There are reports of poor long-term survival of patients with CAD and severe LVD under medical therapy (Cassar et al., 2009; Shapira et al., 1995). Furthermore, the impaired ventricular function can be attributed to declines in perfusion and to unfavorable ventricular remodeling. Ventricular remodeling is induced by a multitude of stimuli, including myocardial infarction, and pressure and/or volume overload. While ventricular remodeling is initially a compensatory response, the transition to adverse remodeling frequently culminates in the development of congestive heart failure (CHF) which significantly contributes towards cardiovascular morbidity and mortality rates (Sutton and Sharpe, 2000).

The renin–angiotensin–aldosterone system (RAAS), matrix metalloproteinases (MMPs), and inflammatory pathways have been shown to be involved in many cardiovascular diseases, including myocardial fibrosis and hypertrophy, congestive heart failure, myocardial infarction, and cardiomyopathy (Deschamps and Spinale, 2006; Hanatani et al., 1995; Kuusisto et al., 2012; Weber et al., 1993). Among the pathways

Abbreviations: ACE, angiotensin I converting enzyme; AT1, angiotensin II type 1 receptor; CART, classification and regression tree; CAD, coronary artery disease; CHF, congestive heart failure; CVC, cross validation consistency; G-score, genotype score; LVD, left ventricular dysfunction; MDR, multifactor dimensionality reduction; MMPs, matrix metalloproteinases; LVEF, left ventricle ejection fraction; LVEDD, left ventricle end diastole dimensions; LVESD, left ventricle end systolic dimensions; RAAS, renin–angiotensin–aldosterone system; RFLP, restriction fragment length polymorphism.

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that contribute to ECM (extracellular matrix) remodeling, the MMPs appear to be of particular interest. MMPs are proteolytic enzymes believed to be involved in many physiological and pathological processes associated with inflammatory reactions (Gunja-Smith et al., 1996; Li et al., 2002; Rutschow et al., 2006; Spinale et al., 1998).

Previously, we have examined the role of individual genetic variations in LVD susceptibility of North Indian CAD patients (Mishra et al., 2012a,b, 2013). However, single SNPs cannot account for susceptibility to complex condition like LVD. In addition, gene–gene and gene environment interactions are believed to be major players in the pathogenesis of such diseases. However, the analysis of such interactions is weighed down by two major problems, one is a small sample size and secondly the curse of dimensionality. Traditional approaches like logistic regression modeling are limited in their ability to deal with many factors and simultaneously fail to characterize epistasis models in the absence of main effects due to the hierarchical model-building process.

Recently, multifactor-dimensionality reduction (MDR) approach (Ritchie et al., 2001), tree-based techniques: classification and regression trees (CART) (Srivastava et al., 2011) and random forest (RF) (Lunetta et al., 2004) and genotype scores (G-score) (de Haan et al., 2012) have been used to detect interactions in large-scale association studies. The strength of these methodologies is their ability to identify association in the cases of small sample sizes and low penetrance of candidate single nucleotide polymorphisms (SNPs). To deal with these issues, we have extended our previous work by jointly investigating 11 SNPs, belonging to RAAS [angiotensin I converting enzyme; *ACE* I/D (rs4340), Ang II type1 receptor; *AT1*A1166C (rs5186), aldosterone synthase; *CYP11B2* T-344C (rs1799998)], MMPs [*MMP2* C-735T (rs2285053), *MMP7* A-181G (rs11568818), *MMP9* R279Q (rs17576), P574R (rs2250889) and R668Q (rs17577)] and inflammatory pathway [*NFKB1-94* ATTG ins/del (rs28362491), *IL6*-174G/C (rs1800795) and *TNF-α*-308G/A (rs1800629)], to achieve a comprehensive evaluation of LVD risk in patients with significant CAD.

2. Materials and methods

2.1. Ethics statement

The study protocol was approved by the institutional ethical committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), and the authors followed the norms of World's Association Declaration of Helsinki (Rickham, 1964).

2.2. Study population

The present study recruited a total of 510 CAD patients and 230 healthy controls of North Indian Ethnicity. Among 510 CAD patients, 162 patients with reduced left ventricular ejection fraction (LVEF $\leq$ 45%) were considered as having LVD while the remaining 348 patients with LVEF > 45% were considered as non-LVD patients. All the patients were recruited from the Department of Cardiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh, India. All the CAD patients had significant coronary artery disease, as confirmed by coronary angiography. Further, all the subjects underwent coronary angioplasty. The echocardiography assessments of the patients were noted just before the coronary angioplasty procedure, irrespective of the myocardial infarction event. The detailed clinical history of CAD patients was based on hospital investigations including coronary angiography. Angiographically identified stenoses >70% in the major coronary vessels at the time of the study were used to classify patients as having single-vessel, double-vessel, or triple-vessel disease. CAD patients having any other cardiac disorders like cardiomyopathies and valvular disease were excluded from the study. Non-LVD population consisted of 348 subjects including 301 males and 47 females with mean age (in years) 56.00 ± 9.88, while LVD population consisted of 162 subjects including 138 males and 24 females with mean age (in years) 56.24 ± 8.51.

The healthy control (non-CAD) population consisted of 230 subjects (197 males and 33 females) (mean age years 54.18 ± 8.47) with no clinical evidence of CAD or LV dysfunction (by echocardiography) and also without a positive family history of CAD or myocardial infarction (MI). Furthermore, the inclusion criteria for controls were the absence of prior history of high systolic blood pressure, abnormal lipid profile, hypertension, obesity and any chronic disease. Both patients and controls were frequency-matched for age, gender and ethnicity. A written informed consent was taken from all the study subjects. After informed consent, all the subjects were personally interviewed for information on food habits, occupation and tobacco usage.

2.3. Data collection

The clinical data were obtained by reviewing the patient's medical records. Left ventricle ejection fractions (LVEFs), left ventricle end diastole dimensions (LVEDDs), left ventricle end systolic dimensions (LVESDs), posterior wall end diastole dimensions and, interventricular septum end diastole dimensions were calculated quantitatively by echocardiography, just before the angiography procedure, using the Simpson's method (Schiller et al., 1989). The LV mass was calculated by using the following formula: $0.8[1.04\{(\text{LV diastolic internal dimension} + \text{interventricular septum} + \text{posterior wall})^3 - (\text{LV diastolic internal dimension})^3\}] + 0.6$ (Devereux et al., 1986). Echocardiography was repeated in 10% of patients and results were totally concordant. The hypertensive patients were defined as having systolic blood pressure > 140 mm Hg or a diastolic blood pressure > 90 mm Hg or using antihypertensive drugs. Smoking was classified as smokers and non-smokers. Similarly, diabetes mellitus was defined as patients with fasting plasma glucose > 6.9 mmol/l or patients using anti-diabetic medication. The height and weight of each subject were recorded to calculate the BMI status, followed by the risk of obesity. All laboratory parameters, as stated in the medical record, were determined in overnight-fasting patients. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were measured by standard enzymatic methods. LDL cholesterol concentrations were calculated using the Friedewald's formula (Johnson et al., 1997).

2.4. DNA samples and genotyping

Genomic DNA was extracted from 5 ml peripheral blood leukocytes according to standard salting out method (Miller et al., 1988). The blood sample and the clinical details were collected from each participant at the time of recruitment. The polymorphisms were genotyped using the PCR/ARMS-PCR or PCR-restriction fragment length polymorphism (RFLP) method as described earlier (Mishra et al., 2012a,b, 2013). As a negative control, PCR mix without DNA sample was used to ensure contamination free PCR product. The digested PCR fragments were separated on 15% polyacrylamide gel, stained with ethidium bromide and observed with ultraviolet imaging system (BIO-RAD Gel-Doc™ EZ imager, PA, USA). Genotyping was performed without the knowledge of the LVD and non-LVD patients. Ten percent of masked, random sample of LVD and non-LVD patients were tested twice by different laboratory personnel and the reproducibility was 100%.

2.5. Statistical analysis

2.5.1. Single locus analysis

Descriptive statistics were presented as mean and standard deviation [SD] for continuous measures while absolute value and percentages were used for categorical measures. Differences in genotype and allele frequencies between study groups were estimated by chi-square test. The ORs were adjusted for confounding factors such as age and gender. All statistical analyses were performed using SPSS software version 16.0

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