Perspectives

Polymorphisms of β-adrenoceptor and Natriuretic Peptide Receptor Genes Influence the Susceptibility to and the Severity of Idiopathic Dilated Cardiomyopathy in a Chinese Cohort

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ABSTRACT

Background: This study evaluated the potential effects of β-adrenoceptor (β-AR) and natriuretic peptide receptor (NPR) gene polymorphisms on the susceptibility to and the severity of idiopathic dilated cardiomyopathy (IDCM) in a Chinese cohort.

Methods and Results: Ten polymorphisms in the coding regions of β1-AR, β2-AR, β3-AR, NPR1, and NPR2 were genotyped in 430 IDCM patients and 468 healthy subjects. Patients with IDCM were followed for 2 years. In multi-loci combined subtype analysis, the combined profile of β-AR and NPR was significantly different between IDCM patients and controls ($P < .0001$), mainly influenced by 2 loci β1-Ser49Gly and NPR2-C2077T, which were also associated with the severity of IDCM. In single-loci analysis, allele frequencies of β1-Gly49, NPR1-Glu939, and NPR2-T2077 were higher in patients with IDCM than in controls. Genotypes carrying NPR2-T2077 allele showed 1.94-fold independent risk for IDCM phenotype than C2077 homozygote ($P < .001$). Carriers of the NPR2-T2077 or β1-Gly49 variant had worse New York Heart Association functional class or echocardiographic results and elevated serum brain natriuretic peptide, experienced severe symptoms, and required intensive medications and frequent hospitalization for heart failure. Furthermore, synergistic interactions between NPR2-C2077T and β1-Ser49Gly were detected by multifactor-dimensionality reduction method.

Conclusions: This study suggests that NPR2-T2077 and β1-Gly49 polymorphisms may be genetically synergistic adverse factors for the susceptibility to or the severity of IDCM. (*J Cardiac Fail 2010;16:36–44*)

Key Words: Idiopathic dilated cardiomyopathy, polymorphism, β-adrenoceptor, natriuretic peptide receptor.

Idiopathic dilated cardiomyopathy (IDCM), a multifactorial disease, is characterized by an enlarged left ventricle and impaired myocardial contractility. It is also a major cause of death in youth with heart failure. Emerging evidence has suggested that genetic factor may play a role in the pathophysiology of IDCM besides viral and immunologic mechanisms.[1–3]

Given the crucial role of adrenergic pathway in myocardial function and marked alternations in β-adrenoceptor (β-AR)-mediated signal transduction in IDCM,[4] previous studies have examined the involvement of β-AR subtype gene polymorphisms in the pathogenesis of cardiac dysfunction, including β1-Ser49Gly, β1-Arg389Gly, β2-Arg16Gly, β2-Gln27Glu, β2-5′LC- Cys19Arg, β2-Thr164Ile, and β3-Trp64Arg.[5–8] Results, however, have not been consistently replicated. In addition, in vitro and in vivo experiments showed that β-AR polymorphisms might have an influence on G protein expression and transportation (β1-Ser49Gly), and associate with downregulated expression of β receptor (β2-Arg16Gly and Gln27Glu), abnormal coupling of β-AR to G protein (β1-Arg389Gly and β2-Thr164Ile), and negative inotropic effect (β3- Trp64Arg).[6–10] These observations further added interesting information on ongoing debates regarding genetic variation in IDCM.

The natriuretic peptide family consists of three peptides, namely atrial natriuretic peptide, brain natriuretic peptide

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(BNP) and C-type natriuretic peptide, which elicits physiologic function via engagement to natriuretic peptide receptor (NPR)1 or NPR2.[11] Recent studies have discovered several polymorphisms in NPR receptor gene (−293 CTn [n=6, 10, 11], Arg939Glu, Met341Ile, 3C/4C at 14319, and I/D [AGAA] at 14649 in 3′-UTR in NPR1; C2077T and 9-bp I/D [gctggggcc] in intron 18 in NPR2).[12−16] Some polymorphisms affected the expression of these receptors and were associated with hypertension, cardiac hypertrophy, and myocardial infarction,[12−16] but their relation to IDCM remains unclear.

In this study, we evaluated the potential association of major polymorphisms in β-AR and NPR subtype genes with the susceptibility to IDCM in a Chinese cohort. The relations of these polymorphisms to disease severity were further investigated during a 2-year follow-up.

Materials and Methods

The study protocol was approved by the local hospital Ethics Committee, and written informed consent was obtained from all participants.

Patients

The study population consisted of 430 patients with IDCM (age, 56±15 years; IDCM duration, 3.5±3.1 years) between January 2000 and May 2006, from 3 teaching hospitals affiliated to the Shanghai Jiaotong University School of Medicine. IDCM was diagnosed according to World Health Organization definition.[17] The patients were capable of having adequate echocardiographic images. To avoid confounding variables, we excluded patients with coronary heart disease, history of hypertension or viral myocarditis, hypertrophic cardiomyopathy, primary valvular disease, and pulmonary heart disease.

Follow-up

All IDCM patients were seen every month in a special clinic of heart failure. During each visit, heart rate, blood pressure, and other clinical manifestations were recorded, and echocardiography was performed every 6 months. Adverse events (hospitalization or death from heart failure) were documented during visit or telephone conversation with patients or their family members. Hospitalization for heart failure was defined as that due to progressive fluid retention and need to increase or change medications. Dyspnea and pedal edema were evaluated.[18−20] Oral diuretic treatment was defined as continuous and long-term administration for at least 3 months. Two trained physicians independently reviewed all medical notes, including emergency department visit forms and hospital medical records.

The severity of IDCM was evaluated according to New York Heart Association functional class, echocardiographic measurements, severe arrhythmias, and adverse events. The response to anti−heart failure therapies, including β-blockers and inhibitors of renin-angiotensin system, was defined as previously reported.[21]

Control Subjects

A total of 468 normal healthy subjects (274 men and 194 women, mean age 52±6 years) served as a control group. Detailed medical and family histories were taken and fasting blood samples collected during an annual physical checkup. None was receiving medications or had history of hypertension, diabetes, or coronary artery disease (including histories of angina/myocardial infarction). Their serum levels of glucose and lipid, liver and renal functional tests, and electrocardiograms were normal.

Biochemical Investigations

Peripheral venous blood samples were collected after an overnight fasting in all participants. Serum glucose, blood urea nitrogen, creatinine, uric acid, total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein cholesterol, lipoprotein (a), apoprotein A, apoprotein B, and triglycerides were measured with standard laboratory techniques on a Hitachi 912 Analyzer (Roche Diagnostics, Germany). Serum BNP-32 was determined using commercially available EIA kit (Phoenix Pharmaceuticals). The linear range for this assay was 0.5 to 100 ng/mL and the maximum interassay coefficient of variation was <4.0%.

Polymerase Chain Reaction and Genotyping

Genome DNA was prepared with a DNA extraction kit (Qiagen) from an EDTA blood sample and stored at -80°C before use. Polymerase chain reaction (PCR) analysis was performed using DNA as a template in a final reaction volume of 50 μL. Genotyping was determined by restriction fragment length polymorphism. Primer3 online software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) was used to design primers. The primers, annealing temperature, restriction enzymes (New England Biolabs), and restriction enzyme digestion map are displayed in Table 1. Briefly, each target sequence was amplified by Taq DNA polymerase (2 units, Boehringer Mannheim) using a pair of primers (15 ng) and genomic DNA (250 ng). PCR conditions were: denaturation at 94°C for 3 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 52 to 65°C for 30 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 10 minutes. PCR products and restriction enzyme digestion map were visualized in 2% agarose gel. The polymorphisms of NPR1-293CTn, 14319Cn, 14649 I/D (AGAA), and NRP2-I/D (gctggggcc) were determined as previously reported.[14−16]

Statistical Analysis

Simple statistical analysis was done using SPSS version 13.0. Continuous variables were compared using *t*-test or 1-way analysis of variance. Chi-square test or Fisher's exact test were run to assess the goodness-of-fit of Hardy-Weinberg equilibrium in control subjects with 1 degree of freedom, as well as to test associations of other categorical variables. A stepwise logistic regression analysis was used to test whether the genetic variant was associated independently with the susceptibility to IDCM after adjustment for age and gender. $P < .05$ was considered statistically significant. The rigorous Bonferroni correction was applied in multiple testing.

The linkage disequilibrium (LD) degree and blocks on the same chromosome were identified by the Haploview software V.4.1 (http://www.broad.mit.edu/mpg/haploview), with LD coefficient shown as D' on the basis of a 4-gamete color scheme.

To test the associations of statistically inferred combined subtypes with the susceptibility to and the severity of IDCM, we used the Haplo.score approach as outlined by Schaid et al,[22] which models an individual's phenotype as a function of each inferred haplotype, weighted by haplotypes estimated probability, to account for haplotype ambiguity. To obtain odds ratios (ORs) of risk haplotypes,