

Brief Report

Association of HLA-DQ with Idiopathic Dilated Cardiomyopathy in a Northern Chinese Han Population

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Autoimmune mechanisms are likely involved in the pathogenesis of idiopathic dilated cardiomyopathy (IDC) and components of MHC may serve as markers for the propensity to develop immune-mediated myocardial damage. This study was conducted to investigate the possible association between HLA-DQA1, -DQB1 alleles and IDC in Han population in northern China by using PCR-based sequence-specific primer (PCR-SSP) technique for HLA genotyping. Among 68 unrelated IDC patients, 4 probands of IDC pedigrees and 100 healthy controls, we found that the alleles of HLA-DQA1*0501 and HLA-DQB1*0303 conferred susceptibility to IDC while DQA1*0201 and DQB1*0502, *0504 alleles were in negative association with IDC. The serine at position 57 (*SER*⁵⁷) in the exon of DQB1*0502 and *0504 was confirmed in our experiment as a marker for resistance to IDC. The results suggest that HLA-DQ polymorphism may be involved in the pathogenesis of IDC. *Cellular & Molecular Immunology*. 2004;1 (4): 311-314.

Key Words: idiopathic dilated cardiomyopathy, HLA-DQA1, HLA-DQB1, polymorphism, genetic susceptibility

Introduction

Idiopathic dilated cardiomyopathy (IDC) is characterized by dilation and impaired contraction of the left ventricle or both, it is a relevant cause of heart failure and a common indication for heart transplantation (1). The major pathogenetic hypothesis in IDC involves autoimmune mediated damage to myocytes. Development of autoimmune inflammatory damage occurs only in patients with a predisposing genetic background. Changes in the immune system concerning cell-mediated and humoral immunity have been detected (2). The immune system is strictly related to human leukocyte antigen (HLA). As in some autoimmune diseases, a relationship between IDC and HLA class II phenotype has been found: particularly the DR4 antigen seems to be associated with a high risk (3, 4). Primary function of HLA is to restrict T-cell receptors in the process of recognizing auto- or exterior antigen, and thus participates in or mediates immunological recognition, immunological response and immune regulation on various levels. HLA is a genetic marker of susceptibility to auto-

immune myocardial damage (5). Previous studies revealed that autoimmune mechanisms are likely to participate in the pathogenesis of at least a subgroup of idiopathic dilated cardiomyopathy and components of the major histocompatibility complex (MHC) may serve as markers for the propensity to develop immune-mediated myocardial damage. HLA class II genes, especially HLA-DQ genes, which are highly polymorphic, play an important role in the activating of immune responses and thus control the predisposition to or protection from IDC. In the present study, we detected the HLA-DQA1 and -DQB1 alleles in IDC patients with the techniques of polymerase chain reaction-sequence specific primers (PCR-SSP) to explore the immunogenetic mechanisms involved in the pathogenesis of IDC.

Materials and Methods

Study subjects

This case-control study was carried out within 68 unrelated IDC patients (48 males and 20 females) recruited from the 1st. affiliated hospital of Harbin Medical University during the period of 1999-2002. The ages ranged from 17 to 67 with mean age of 46.7 ± 12.3 years. The clinical diagnosis was made according to World Health Organization criteria (IDC group). Four probands of IDC pedigrees (3 males and 1 female), whose average age was 39.0 ± 8.0 years, were included in IDC group. The control group consisted of 100 unrelated healthy subjects (62 males and 38 females) from routine health survey, whose ages ranging from 27 to 59

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Abbreviation: HLA, human leukocyte antigen; IDC, idiopathic dilated cardiomyopathy; MHC, major histocompatibility complex; PCR-SSP, polymerase chain reaction - sequence specific primers; SLE, system lupus erythematosus; RA, rheumatoid arthritis; EF, ejection fraction.

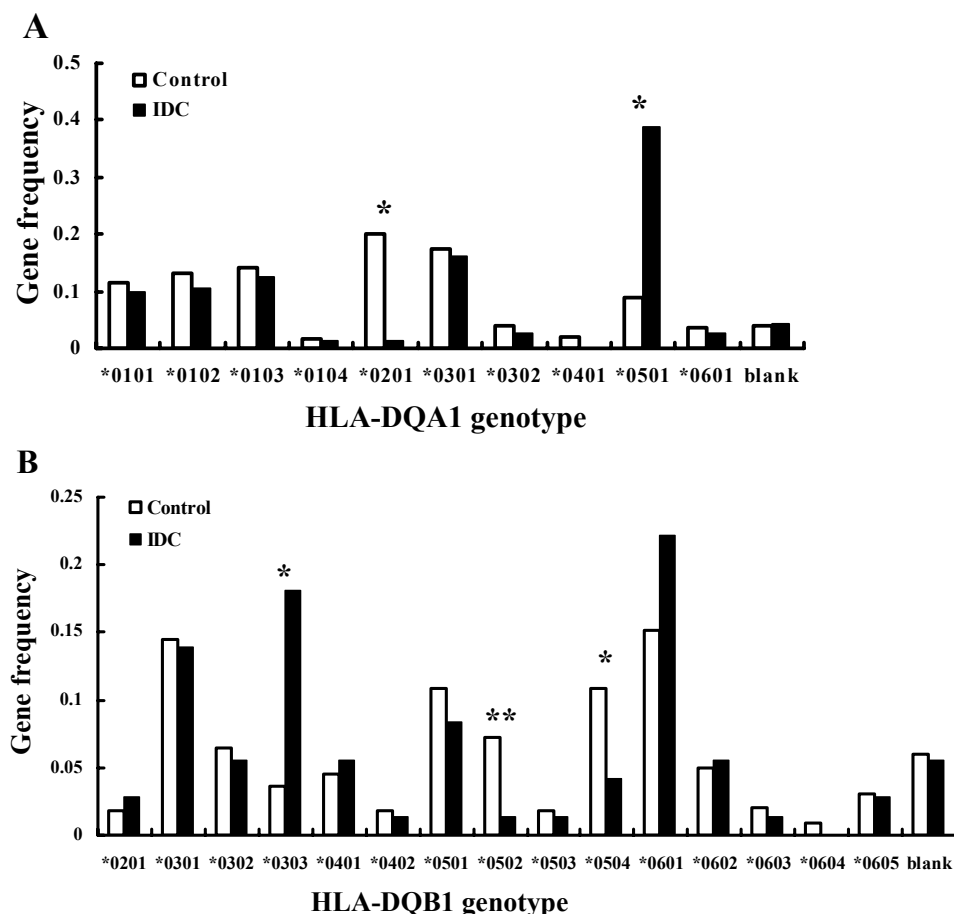


Figure 1. Comparisons of allele frequencies of HLA-DQA1 (A) and -DQB1 (B) in the two groups. Genomic DNA was extracted from peripheral blood. The polymorphisms of HLA-DQA1 and -DQB1 alleles among 68 unrelated IDC patients, 4 probands of IDC pedigrees and 100 healthy controls were analyzed using polymerase chain reaction sequence - specific primer (PCR-SSP) technique. * $p < 0.05$ vs the control group; ** $p < 0.01$ vs the control group.

with mean age of 51.1 ± 16.3 years. All subjects were from Heilongjiang province of Northern China and of the Han nationality. Patients with a history of heavy alcohol intake, systemic diseases of putative autoimmune origin or skeletal myopathies were specially excluded. All individuals with HLA-related disorders, such as SLE and RA, were also excluded in this investigation.

Echocardiographic studies were performed in the IDC group and such group was stratified according to ejection fraction (EF). Those whose EF values were higher than 35% were subjected into subgroup 1. Subgroup 2 included the patients with an EF value between 15% and 35%, and subgroup 3 consisted of those whose EF values were lower than 15%.

HLA-DQA1, -DQB1 genotyping

Genomic DNA was extracted by phenol-chloroform-isoamyl from peripheral blood with the method as previously described (3). The special oligonucleotide primers for the second exon of HLA-DQA1 and -DQB1 genes were synthesized by the Central Research Laboratory of the People's Hospital, Peking University as described by Olerup (4). Primers included nucleotides GAT or GAC which identify aspartic acid (ASP) located on DQB chain (ASP^{57}), or GCC, ACG, GTT which encode ALA (ALA^{57}), or sections that encode SER (SER^{57}) and VAL (VAL^{57}). All alleles could be identified and all homozygous and heterozygous combinations were distinguishable.

Each PCR reaction included a primer pair amplifying the third intron of DRB1 gene. These two primers matched non-allelic sequences and thus functioned as an internal positive amplification control. 5'-primer C5 and 3'-primer C3 gave rise to a 796-base pair fragment. The second exon of the DQA1 and DQB1 gene were amplified by PCR-SSP. The reaction system (15 μ l) included DNA 75 ng, PCR buffer 1.5 μ l, dNTPs (25 mmol/l) 0.6 μ l, specific primers (5 pmol/l) 1.0 μ l, and 0.5 U of the Taq DNA polymerase (Promega). In addition, the internal positive control primer pair, C3 (5'-GCA TCT TGC TCT GTG CAG AT-3', complementary to codons 193-200 in the 5'-end of exon 4) and C5 (5'-TGC CAA GTG GAA CAC CCA A-3', complementary to codons 173-179 in the 3'-end of exon 3), was included in all reaction mixtures in a 5-fold lower concentration than the allele- and group-specific primers. The reaction mixture was subjected to 35 amplification cycles, each consisting of denaturation at 94°C (30 s), annealing at 58°C (30 s) and extension at 72°C (60 s) with a final extension step of 180 s at 72°C. PCR products were visualized by agarose gel electrophoresis. After addition of 2 μ l loading buffer, the PCR reaction mixtures were loaded in agarose gels pre-stained with ethidium bromide (0.5 μ g/ml gel). Gels were electrophoresed for 15 minutes at 10 V/cm in 0.5 $\times$ TBE (Tris-Boric acid-EDTA) buffer, then examined under UV illumination and documented by photography.

Statistics

HLA-DQ alleles were determined by direct counting. Allelic frequency was calculated as follows: allelic frequency = $n/2N$, where n represented the number of individuals carrying this allele, and N for total number of subjects. Comparisons of allelic frequencies were performed using χ^2 on the computer, and p values < 0.05 were considered significant difference. The strength of an association was expressed by odds ratios (ORs) with 95% confidence interval (95% CI). Linkage analysis between IDC pedigree and polymorphic gene loci was analyzed with GENEHUNTER software.

Results

The frequencies of HLA-DQA1, -DQB1 alleles differently distribute in IDC patients and healthy control

The distribution of HLA-DQA1 and -DQB1 alleles in the two groups is in accordance with the Hardy-Weinberg hereditary equilibrium law. The HLA-DQA1 and -DQB1 allele frequencies of the subjects were presented in Figure 1. Carrying 56 HLA-DQA1*0501 and 26 HLA-DQB1*0303 alleles among the total 72 patients, HLA-DQA1*0501 (0.3889 vs 0.0900, $p < 0.05$) and DQB1*0303 (0.1806 vs 0.0364, $p < 0.05$) alleles were significantly increased in IDC group than those in the healthy controls. Further analysis of the three subgroups showed a higher frequency of DQA1*0501 and DQB1*0303 among patients in subgroup 3 with lower EF values ($p < 0.05$). Increased frequency of DQA1*0201 (0.2000 vs 0.0139, $p < 0.05$), DQB1*0502 (0.0727 vs 0.0139, $p < 0.01$) and DQB1*0504 (0.1091 vs 0.0417, $p < 0.05$) were found in the control group compared with the IDC group.

HLA-DQA1 controls the predisposition to IDC

IDC patients carrying the HLA-DQA1*0501 allele had more serious clinical manifestations and lower EFs ($p < 0.01$). No differences were found in EFs among patients with different HLA-DQB1 alleles.

The distributions of HLA-DQA1, -DQB1 are associated with IDC disease

General data of IDC pedigrees was that 26 people were in the 4 IDC families, male: female = 11:15, with a mean age of 39.0 ± 8.0 years. There were totally 15 IDC patients in the 4 pedigrees (57.7%), including 11 male (73.3%) and 4 females (26.7%). Four probands included 3 male and 1 female. Distributions of HLA-DQA1*0501 and HLA-DQB1*0303 alleles in the 4 families were 0.4013 and 0.2680, respectively and they were the highest in the 4 families. The linkage Lod values of HLA-DQA1, -DQB1 with IDC pedigree was less than 1 by linkage analysis.

Discussion

IDC is characterized by increased left ventricular intracavitary dimensions, reduced systolic function, and a congestive heart failure syndrome (6, 7). Without a clear-cut etiology, IDC is a tough problem in the cardiovascular medical practice. The heart failure resulting from IDC has some different characteristics from those

resulting from rheumatic heart disease or coronary heart disease, etc (8). Clinical heterogeneity seems to imply a peculiar genetic background. Limas, et al. found that autoimmune mechanisms are likely to participate in the pathogenesis of at least a subgroup of dilated cardiomyopathy and components of MHC may serve as markers for the propensity to develop immune-mediated myocardial damage (9). Autoimmune features in patients with myocarditis/IDC include: familial aggregation, a weak association with HLA-DR4, abnormal expression of HLA class II on cardiac endothelium on endomyocardial biopsy, and detection on organ- and disease-specific cardiac autoantibodies, by immunofluorescence and absorption techniques, in the affected patients and in a proportion of their symptom-free relatives from both familial and non-familial IDC pedigrees. These suggest that autoimmune mechanisms under the control of the class II genes might play an important role in the pathogenesis of IDC.

Genes linked to HLA-alleles such as DR4 may contribute to the etiologic process of cardiac damage (1, 10). Observations have also implicated that HLA-DQA1 and -DQB1 genes are in the susceptibility to presumed autoimmune diseases such as diabetes mellitus, multiple sclerosis and scleroderma (11). In present study, HLA-DQA1 and -DQB1 loci were genotyped with PCR-SSP and the frequencies of DQA1*0501, DQB1*0303 were found to be increased among IDC patients, indicating they were susceptible genes to the development of IDC. This conclusion was substantiated by the trend of increasing frequency DQA1*0501 and DQB1*0303 with EF declination after stratification. However, HLA-DQA1*0201 and HLA-DQB1*0502, *0504, which had higher gene frequencies in normal controls, manifested lower distribution in IDC group, suggesting that HLA-DQA1*0501 and DQB1*0303 were related to the genetic susceptibility to IDC while DQA1*0201, DQB1*0502 and DQB1*0504 alleles conferred protection from IDC. Perhaps the alleles or linkage disequilibrium between these alleles and other HLA loci influence the T cell immunity involved in myocardial damage. The SE^{57} in the exon of DQB1*0502 and *0504 might confer resistance to IDC, defect or substitute of this amino acid residue at position 57 of DQ β chain might lead to IDC susceptibility. HLA-DQ allele polymorphisms may serve as genetic markers for IDC and be involved in the regulation of immune specific response to auto- or exterior anti-myocardium antibody.

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