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Association of IL-4 gene polymorphisms with Ischemic Heart Failure

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Running Title: IL-4 SNPs in Ischemic Heart Failure

Abstract

Background: As of the potential immunomodulatory effects of interleukin-4 (IL-4) and its importance in inhibiting the production of proinflammatory cytokines by monocytes and activated T cells, the IL-4 gene polymorphisms were investigated in a group of patients with chronic heart failure (CHF) due to ischemic heart disease.

Methods: Forty three patients with ischemic heart failure were enrolled in this study and compared with 139 healthy individuals. The allele and genotype frequency of 3 single nucleotide polymorphisms (SNPs) within the IL-4 gene were determined.

Results: The frequency of the IL-4 -590/T allele in the patient group was significantly higher than in the control group ($p < 0.0001$). The most frequent genotypes in patients with ischemic heart failure were IL-4 (-590) CC ($p < 0.0001$), IL-4 (-33) CC ($p=0.021$), and IL-4 (-33) TT ($p < 0.0001$). The frequency of the following genotypes was significantly lower in patients compared to controls: IL-4 (-1098) TG ($p=0.035$), IL-4 (-590) TC ($p < 0.0001$), and IL-4 (-33) TC ($p < 0.0001$). The most frequent IL-4 haplotypes in the patient group, which were significantly higher than in the control group, were TCC ($p < 0.0001$), TCT ($p=0.0242$), and GCT ($p=0.0108$) haplotypes. In contrast, the frequencies of the following haplotypes in the patient group were significantly lower than the controls: GCC ($p=0.032$), TTT ($p=0.0268$), and TTC ($p=0.0399$).

Conclusions: Certain alleles, genotypes, and haplotypes in IL-4 gene were overrepresented in patients with ischemic heart failure, which may, in turn, predispose individuals to this disease.

Key words: Heart failure, gene polymorphisms, interleukin-4

Introduction

Chronic heart failure (CHF) is a clinical syndrome characterized by progressive ventricular dilation, depressed contractile function, and increased morbidity and mortality . Despite our increasing knowledge of the molecular, genetic, and biochemical pathways involved in the pathogenesis of CHF, much is left to be understood.

Increased circulating and intracardiac levels of proinflammatory cytokines have been reported repeatedly in patients with CHF , and more important, their contributions in mediating cardiac adaptive and maladaptive responses have been a topic of intensive research .

Given the potential immunomodulatory effects of IL-4 and its importance in inhibiting the production of proinflammatory cytokines by monocytes and activated T cells , IL-4 gene polymorphisms altering cytokine production might affect individual susceptibility to ischemic heart failure. The association of IL-4 gene polymorphisms and a number of diseases with possible underlying immune disturbances have already been investigated . In the present study, three cytokine single nucleotide polymorphisms situated at positions -1098 (G/T), -590 (C/T), and -33 (C/T) in the promoter region of the IL-4 gene were investigated in Iranian patients with ischemic heart failure.

Materials and Methods

Subjects

In the present study, a total of 43 Iranian patients with end-stage ischemic heart failure (mean age 60.05 ± 11.97 ; 34 men, 9 women) with angiographically significant coronary artery disease, defined as $\geq 50\%$ diameter stenosis in at least one of the major coronary arteries, were enrolled. The diagnosis of end-stage heart failure was based on impaired left ventricular systolic function (left ventricular ejection fraction $\leq 40\%$) and left ventricular dilation (left ventricular end-diastolic diameter > 5.5 cm) on echocardiography. All patients underwent transthoracic echocardiography and cardiac catheterization. Subjects with chronic lung disease, malignancies, recent myocardial infarction, and acute decompensated heart failure within 3 months before recruitment were excluded. Eligible patients were in stable clinical condition and received conventional medical therapy for at least 3 months.

One hundred and thirty nine control subjects (mean age 45.63 ± 10.84 ; 101 men, 39 women) were randomly selected from healthy volunteers, as previously described . Written informed consent was obtained from all participants prior to blood sampling. This study was approved by the Ethical Committee of Tehran University of Medical Sciences.

Genotyping

Cytokine typing was performed on genomic DNA by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as previously described in detail . Briefly, amplification was carried out using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. All individuals were genotyped for 3 polymorphic sites in IL-4 gene: -1098 T/G, -590 C/T, and -33 C/T.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism 5.00 for Windows (Graphpad Software). Allele, genotype, and haplotype frequencies for all cytokine gene polymorphisms were calculated by direct counting. Frequencies of alleles, genotypes, and haplotypes were compared between the patient and control groups using the Fisher's exact test. The odds ratio and 95% confidence intervals were calculated.

Results

Alleles and Genotype Frequencies

IL-4 allelic and genotype frequencies in patients with ischemic heart failure and healthy controls are presented in Table 1.

The frequency of the IL-4 -590/T allele in the patient group was significantly higher than in the control group (73.3% in patients vs. 46.4% in controls, $p < 0.0001$). The most frequent genotypes in patients with ischemic heart failure were IL-4 CC genotype at position -590 (51.1% in patients vs. 7.2% in controls, $p < 0.0001$), IL-4 CC genotype at position -33 (65% in patients vs. 43.9% in controls, $p=0.021$), and IL-4 TT genotype at position -33 (15% in patients vs. 0% in controls, $p < 0.0001$). In contrast the frequency of the following genotypes in the patient group was significantly lower than in the control group: IL-4 TG at position -1098 (39.6% in patients vs. 59% in controls, $p=0.035$), IL-4 TC at position -590 (44.2% in patients vs. 92.8% in controls, $p < 0.0001$), and IL-4 TC at position -33 (20% in patients vs. 56.1% in controls, $p < 0.0001$).

Haplotype Frequencies

The frequency of haplotypes of IL-4 (-1098, -590, -33) in patients with ischemic heart failure and healthy controls are shown in Table 1. The most frequent haplotype in our patients was IL-4 TCC which was significantly higher than in the control group (47.5% in patients vs. 23.4% in controls, $p < 0.0001$). In addition IL-4 TCT (5% in patients vs. 0.7% in controls, $p=0.0242$) and GCT (3.75% in patients vs. 0% in controls, $p=0.0108$) haplotypes were significantly more common in the patient group compared to controls. In contrast, the frequencies of the following haplotypes in the patient group were significantly lower than the controls: IL-4 GCC (17.5% in patients vs. 30% in controls, $p=0.032$), TTT (15% in patients vs. 27.3% in controls, $p=0.0268$), and TTC (8.75% in patients vs. 18.3% in controls, $p=0.0399$).

Discussion

In the present study, the frequency of the IL-4/C allele at position -590 was decreased significantly in patients with ischemic heart failure; however, the frequency of the CC genotype at the same position was significantly overrepresented in patients compared to controls. In addition, while less than half of patients had the TC genotype at position -590, it was found in the majority of the healthy controls. It has been shown that the C to T exchange at position -590 of the IL-4 gene is associated with increased IL-4 production, with the CC, TC, and TT genotypes being associated with low, intermediate, and high IL-4 production, respectively . With regard to IL-4 -590 C/T polymorphism, Bijlsma FJ et al. reported no correlation between IL-4 promoter gene polymorphism at position -590 and heart failure, and allograft rejection after heart transplantation. However, in patients with the CC genotype (low IL-4 production), receiving a heart from a T-positive donor reduced the incidence of rejection substantially. It has been concluded that high IL-4 production within -590/T-positive donor hearts protects against rejection .

For IL-4 at position -33, there was no significant difference in allele frequencies between our patients and controls. However, we found significant positive associations with the CC and TT genotypes in our patients compared to controls; while the TC genotype at the same position was decreased significantly in patients than controls. It has been shown that IL-4 promoters harboring -33/C allele plays a significant role in the activation of IL-4 transcription, and that the homozygous variant of IL-4 (-33) CC is associated with increased IL-4 production . In this study, the CC genotype at position -33 was the most frequent genotype in both groups of patients and controls; however, while none of the healthy controls were found to have the TT genotype, it was significantly overrepresented in patients with ischemic heart failure.

The IL-4 (-1098, 0590, -33) haplotypes GCC, TTT, TCC, and TTC are the most common haplotypes in normal Iranian population . In the present study, the TCC haplotype was the most frequent haplotype in patients with ischemic heart failure and was significantly overrepresented in patients compared to controls. However, the GCC, TTT, and TTC haplotypes in the patient group were significantly less

frequent than in healthy controls. On the other hand, the IL-4 TCT and GCT haplotypes were significantly overrepresented in patients than controls. Given the fact that IL-4 (-1098) T/G polymorphism was found to have no correlation with serum IL-4 level , and that the IL-4 -590/C and -33/T alleles are associated with decreased IL-4 production , it could be suggested that there is a tendency towards lowered IL-4 production in at least a subset of patients with ischemic heart failure. However, given the small number of patients in our study, we must view these results with caution.

IL-4 is an important mediator of T-helper 2 (Th2) immune responses by inducing differentiation of naive CD4⁺ T cells to Th2 cells , regulating humoral immune responses , and inhibiting the production of Th1 cytokines by monocytes and activated T cells . Moreover, IL-4 stimulates fibroblast proliferation and collagen synthesis, and thus has been implicated in the progression of fibrosis . Increased urinary excretion of circulating procollagen type-III amino-terminal propeptide (PIIINP) has been known as a marker of ongoing fibrotic processes . Likewise, increased serum PIIINP levels have also been demonstrated in patients with ischemic and dilated cardiomyopathy . It has been demonstrated that IL-4, most likely produced by mast cells in the heart during pressure overload, significantly contribute to cardiac fibrosis . In patients with heart failure, the urinary IL-4 level correlated with left ventricular end-systolic volume index, left ventricular end-diastolic volume index, and with PIIINP . The correlation between urinary IL-4 level and cardiac fibrosis and remodeling was found to be stronger in patients with hypertensive cardiomyopathy . At the present time, the pathologic significance of IL-4 in the development and progression of chronic heart failure is not well understood. The majority of studies failed to demonstrate any significant difference in IL-4 producing peripheral CD4⁺ T cells and IL-4 levels between the two groups of patients and controls .

To the best of our knowledge, we demonstrated for the first time the association between certain allele, genotype, and haplotype frequencies in IL-4 gene with ischemic heart failure. Further studies will provide additional understanding of the possible role of cytokine gene polymorphisms in the development and progression of ischemic heart failure.

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Table 1. Comparisons of allele, genotype and haplotype frequencies between patients with ischemic heart failure and controls.

Cytokine	Position	Alleles/ Genotypes/ Haplotypes	Patients (n=43) N (%)	Controls (n=139) N (%)	P-value	Odds Ratio (95% CI)
IL-4	-1098	G	19 (22.1)	84 (30.2)	0.171	0.65 (0.37 to 1.16)
		T	67 (77.9)	194 (69.8)	0.171	1.53 (0.86 to 2.70)
		GG	1 (2.3)	1 (0.7)	0.418	3.29 (0.20 to 53.71)
		TG	17 (39.6)	82 (59)	0.035*	0.45 (0.23 to 0.91)
		TT	25 (58.1)	56 (40.3)	0.053	2.06 (1.03 to 4.12)
	-590	C	23 (26.7)	149 (53.6)	< 0.0001*	0.32 (0.19 to 0.54)
		T	63 (73.3)	129 (46.4)	< 0.0001*	3.16 (1.86 to 5.39)
		CC	22 (51.1)	10 (7.2)	< 0.0001*	13.51 (5.61 to 32.53)
		TC	19 (44.2)	129 (92.8)	< 0.0001*	0.06 (0.03 to 0.15)
		TT	2 (4.7)	0 (0)	0.055	16.81 (0.79 to 357.3)
	-33	C	60 (75)	200 (71.9)	0.670	1.17 (0.66 to 2.07)
		T	20 (25)	78 (28.1)	0.670	0.86 (0.48 to 1.51)
		CC	26 (65)	61 (43.9)	0.021*	2.38 (1.14 to 4.93)
		TC	8 (20)	78 (56.1)	< 0.0001*	0.20 (0.08 to 0.45)
		TT	6 (15)	0 (0)	< 0.0001*	52.57 (2.89 to 956.5)
IL-4	-1098, -590, -33	GCC	14 (17.5)	83 (30)	0.032*	0.5 (0.27 to 0.94)
		TTT	12 (15)	76 (27.3)	0.027*	0.47 (0.24 to 0.91)
		TCC	38 (47.5)	65 (23.4)	< 0.0001*	2.97 (1.76 to 4.98)
		TTC	7 (8.75)	51 (18.3)	0.039*	0.43 (0.19 to 0.98)
		TCT	4 (5)	2 (0.7)	0.024*	7.26 (1.31 to 40.43)
		GTT	1 (1.25)	1 (0.3)	0.397	3.51 (0.22 to 56.73)
		GCT	3 (3.75)	0 (0)	0.011*	25.15 (1.29 to 492.6)
		GTC	1 (1.25)	0 (0)	0.223	10.51 (0.42 to 260.7)