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Original Article

Correlation between interleukin-4 gene polymorphism and systemic lupus erythematosus and lupus nephritis

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Abstract: Systemic lupus erythematosus (SLE) is a common autoimmune connective tissue disease that may involve a variety of organ functions and seriously affects human health. We investigated interleukin-4 (IL-4) rs2243250 locus polymorphism to explore its correlation with the susceptibility of SLE and lupus nephritis (LN). A total of 186 SLE patients were treated as an experimental group matched with age and gender. Another 186 healthy subjects were selected as the control group. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to test the polymorphic loci and analyze gene distribution. The SLE patients were divided into LN group and non-LN group to investigate the gene distribution. The allele frequency distribution of the IL-4 gene rs2243250 locus was consistent with the genetic Hardy-Weinberg equilibrium law ($P>0.05$). The frequency of the rs2243250 locus and allele in the case group and control group were significantly different ($P<0.05$). The risk of TT genotype carriers was clearly higher than that of CC gene carriers ($P<0.05$). The risk of T allele carriers was higher than that of C allele ($P<0.05$). The occurrence risk of allele and genotype distribution in LN and non-LN groups was markedly different ($P<0.05$). The risk of TT+CT genotype carriers was higher than that of CC gene carriers ($P<0.05$). The IL-4 rs2243250 gene polymorphism was associated with SLE and LN susceptibility. Homozygous TT genotype may be a susceptibility factor of SLE. The TT+CT genotype was a susceptibility factor of LN.

Keywords: Single nucleotide polymorphism, IL-4, SLE, LN

Introduction

Systemic lupus erythematosus (SLE) is a multi-organ and multi-system autoimmune connective tissue disease, which presents as immune system abnormalities, complement activation, autoantibody production, and immune complex deposition. There are gender and racial differences in the incidence of the disease, which mainly occurs in women of childbearing age. Its incidence in the European population is low [1]. Its prevalence in China is higher than that in Japan and other countries [2]. SLE patients are characterized as having high risk of mortality, while LN is one of the main causes of death and can seriously affect the human body and mind health [3].

The incidence of SLE is affected by a variety of factors, thus it is a complex multi-factor disease with unclear pathogenesis [4]. At present, it is thought that the incidence of SLE is caused by genetic factors and non-genetic factors. SLE has a significant family occurrence, with an inheritance potential of 43.6% [5], indicating that the genetic factors play an important role in its pathogenesis. In addition, environmental factors also affect the incidence of SLE, such as viruses and bacterial infections, dietary factors, heavy metal pollutants, etc. [4].

Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by single nucleotide variation. A large number of studies have found that susceptibility to this disease is associated with SNPs. It was observed that SLE susceptibility is associated with genetic polymorphism [6, 7]. IL-4 is a pleiotropic cytokine produced by Th2 cells, basophils, natural killer cells, and mast cells, and is stimulated by Th2 cells [8]. IL-4 plays an important role in the human immune system by promoting the proliferation of B cells and T cell antibodies. It was found that IL-4 serum levels significantly

Table 1. Clinical characteristics of enrolled participants

	Experimental group (SLE)	Control group
n	186	186
Gender (M/F)	37/149	40/146
Age (Average)	40.14±4.56	40.26±4.83
LN (M/F)	102 (20/82)	
Non-LN (M/F)	84 (17/67)	

changed in the morbidity and activity of SLE [9, 10]. It was shown that the IL-4 rs2243250 allele T was related to IL-4 serum level elevation [11]. At present, there is still lack of study about the relationship between this location polymorphism and SLE susceptibility. Therefore, this research conducted a case-control study to clarify the relationship between IL-4 rs2243250 polymorphism and susceptibility to SLE. In addition, it was revealed that IL-4 serum level was significantly correlated with the incidence of LN [12]. Therefore, this study also analyzed the relationship between IL-4 rs2243250 site and LN susceptibility to identify the significance of locus polymorphisms in the pathogenesis of SLE and LN.

Materials and methods

Main instruments and reagents

DNA extraction kit (Applied Biosystems), PCR kit (Takara), AvaII endonuclease (MEB, USA), primers (synthesized by Generay), 2720 PCR Amplifier (ABI), 22331 hamburg protein nucleic acid analyzer (Eppendorf), gel imaging analyzer (Alpha Innotech).

Subjects

A total of 186 SLE patients between January 2014 and December 2016 were enrolled from Yantaishan hospital (Shandong, China). Another 186 healthy subjects were selected as the control group by age and gender matching. SLE and LN patients were diagnosed according to the SLE classification criteria revised by the American College of Rheumatology (ARC) in 1997 [13]. All subjects were of the Han population without blood relationship. Exclusion criteria: 1) rheumatoid arthritis and other immune diseases; 2) family history of immune disease; 3) severe liver and kidney disease; 4) infectious diseases. All subjects signed an informed consent. Venous blood was extracted and stored at -80°C for the study. This study was approved by the medical ethics committee of Yantaishan hospital (Shandong, China).

Peripheral blood DNA extraction

The DNA of peripheral blood cells was extracted by a genomic DNA extraction kit according to the manual. DNA purity and concentration were determined by a protein nucleic acid analyzer. Agarose gel electrophoresis was performed.

IL-4 gene SNP detection

The rs2243250 locus of the IL-4 gene was genotyped by PCR-RELP. The target gene was amplified by a PCR kit (Takara). The reaction system contained 0.25 μg DNA, 2.5 μl 10× buffer, 0.125 μl Taq enzyme, 0.5 μl primer, 2 μl dNTP, and ddH_2O. The conditions were predenaturation at 94°C for 5 min; followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and 72°C for 60 sec. The primers were 5-TAAACTTGGGAGAACATGGT-3 and 5-TGGGGAAAGATAGAGTAATA-3. A total of 5 μL PCR product was used for agarose gel electrophoresis to determine the amplification. A total of 8 μL PCR product was digested by 1 μL AvaII endonuclease and ddH_2O. After incubation at 37°C for 2 h, 10 μL digested product was electrophoresed to determine the rs2243250 genotype. In order to verify the PCR-RELP results, several PCR products were randomly selected for DNA sequencing.

Statistical analysis

SPSS 20.0 statistical software was used for statistical analysis. The continuous variables were expressed as mean ± standard deviation and tested for Hardy-Weinberg equilibrium using χ^2 test. The comparison between the two groups was based on χ^2 test or t-test. Significance level was α=0.05.

Results

Clinical information

There were 37 males and 149 females with an average age of 40.14±4.56 years in the experimental group. There were 40 males and 146 females in the control group with a mean age of 40.26±4.83 years old (t=0.25, P=0.40) (**Table 1**). Chi-square test revealed that the age distribution in two groups lacked statistical difference (χ^2=0.15, P=0.70). There was no signifi-

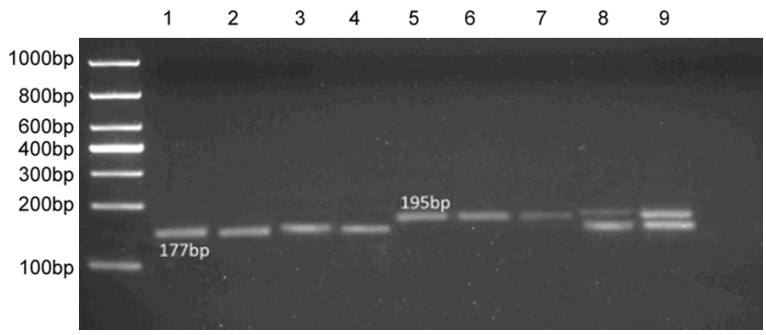

Figure 1. The digestion product of IL-4 rs2243250. 1-4, genotype CC; 5-7, genotype TT; 8, 9, genotype CT; 10, negative control.

cant difference between the two groups (P>0.05). Among SLE patients, there were 102 patients with LN and 84 patients with non LN (**Table 1**). There were 20 males and 82 females with an average age of 40.16±4.46 years old in LN group, while 17 males and 67 females with an average age of 40.12±4.59 years old in non-LN group. No statistical difference was observed in age between the two groups (t=0.02, P=0.49). Chi-square test demonstrated that no significant difference was found in age distribution between the two groups (χ^2=0.01, P=0.91).

Genotype detection

There are three genotypes in the rs22432-50 loci of the IL-4 gene, including TT, CT, and CC. The digestion product genotyping results are shown in **Figure 1** and the sequencing results are revealed in **Figure 2**. The sequencing results were in accordance with PCR-RELP results.

Polymorphism Hardy-Weinberg equilibrium in IL-4 gene rs2243250 loci

Chi-square test demonstrated that the allele frequency distribution of the SNP locus in the control group and the case group was in accordance with the genetic Hardy-Weinberg equilibrium law (P>0.05), indicating that there was no significant difference between the selected population and the general population (**Table 2**).

Genotype polymorphism comparison in SLE patients

The genotype distribution of the two groups is shown in **Table 2**. The genotype distribution of the two groups was statistically different (χ^2=7.12, P=0.03). The risk of having SLE in the TT genotype carriers was clearly higher than in the CC allele group (OR=2.90, 95% CI=1.16~7.25). The frequency of C allele distribution was 82.8% in the case group, which was markedly higher than that in the control group (χ^2=7.69, P=0.01). The risk of having the T allele was apparently higher than that of C allele (OR=1.65, 95% CI=1.16~2.36).

Gene polymorphism comparison between LN and non-LN groups

The genotype distribution of the LN group and non LN group is exhibited in **Table 3**. The genotype distribution of the two groups was statistically different (χ^2=6.27, P=0.04). The risk of having LN in the TT+CT genotype carriers of was clearly higher than that of the CC gene carriers (OR=2.11, 95% CI=1.16~3.85). The frequency of having the C allele in the non LN group was 81.0%, which was markedly higher than that in LN group (χ^2=6.79, P=0.01). The risk of being a T allele carrier was apparently higher than that of group C (P<0.01) (OR=1.90, 95% CI=1.17~3.09).

Discussion

Ramos et al. indicated that “the gene loads ammunition to the gun, the environment pulled the trigger”, which can explain the relationship between environment, genesis, and disease [14]. The pathogenesis of a disease is affected by the environment and genetic interactions, in which genetic susceptibility generally includes chromosome structure and number of abnormalities, single or multiple gene abnormalities, and mitochondrial abnormalities. Gene polymorphism plays an important role in the susceptibility to disease. It is widely used to explore the cause of the disease because of its characteristics of wide distribution, genetic stability, and huge number. It is reported that a large number of interleukin gene polymorphisms are associated with susceptibility to SLE, such as IL-6 [15], IL-33 [16], IL-19 [17], and IL-17 [18]. However, there is still lack of evidence about the relationship between IL-4 rs2243250 polymorphism and SLE susceptibility.

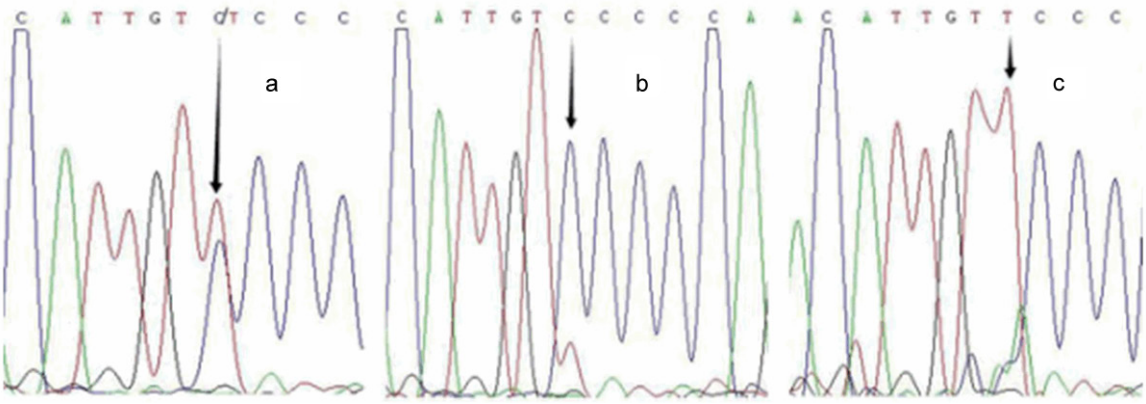

Figure 2. IL-4 rs2243250 loci sequencing result. a, CT; b, CC; c, TT.

Table 2. IL-4 gene rs2243250 locus genotype Hardy-Weinberg equilibrium test

Group	Genotype			Allele		χ^2 value	*P* value
	TT (%)	CT (%)	CC (%)	T (%)	C (%)		
Experimental group (SLE)	17 (9.1)	61 (32.8)	108 (58.1)	95 (25.5)	277 (74.5)	3.53	0.06
Control	7 (3.8)	50 (26.9)	129 (69.4)	64 (17.2)	308 (82.8)	0.59	0.44

Table 3. Genotype distribution between LN and non-LN groups

Group	Genotype			Allele	
	TT (%)	CT (%)	CC (%)	T (%)	C (%)
LN Group	12 (11.8)	39 (38.2)	51 (50.0)	63 (30.9)	141 (69.1)
Non-LN Group	5 (6.0)	22 (26.2)	57 (67.9)	32 (19.0)	136 (81.0)

We enrolled SLE patients and healthy volunteers to investigate the IL-4 SNP. The results of genotyping showed that the allele and genotype of IL-4 rs2243250 were different between the two groups. The risk of having SLE was significantly higher in the TT genotype carriers than that of the CC gene carriers (P<0.05). The risk of being a T allele carrier was clearly higher than that of C allele (P<0.05), suggesting that the allele T and TT genotype may be potential risk factors of SLE. Previous research revealed that the rs2243250 polymorphism is associated with serum levels of IL-4 [11]. Serum IL-4 level also clearly changed in SLE patients [19], revealing that the rs2243250 locus may affect the incidence of SLE by influencing IL-4 serum level. Mohammadoo-Khorasani M et al. found that IL-4 VNTR polymorphism is associated with the pathogenesis of SLE [20]. However, there is still lack of reporting about the relationship between IL-4 rs2243250 polymorphism and SLE susceptibility.

Further analysis of the relationship between IL-4 rs2243250 polymorphism and LN demonstrated that the SLE risk of TT+CT genotype carriers was markedly higher than CC gene carriers, while T allele carriers was apparently higher than that of C allele carriers, suggesting that TT+CT genotype and the T allele may be risk factors for LN. The level of IL-4 in patients with LN was also significantly altered [21]. Thus, the rs2243250 locus may affect the incidence of LN by impacting IL-4 serum levels. Further studies are needed to confirm.

This study explored the relationship between IL-4 rs2243250 polymorphism and SLE and LN susceptibility. The results suggested that the polymorphism of this locus is related to the pathogenesis of SLE and LN, and provided clues to investigate the pathogenesis of SLE. However, this study has some limitations, as a larger scale study is needed in future research to confirm their relationship. In addition, the occurrence of a disease is the result of multiple factors constructed in a huge network. SNP locus research is only one of these factors. Therefore, continued endeavors are needed to clarify the pathogenesis of SLE.

Conclusion

The IL-4 rs2243250 gene polymorphism was associated with SLE and LN susceptibility. Ho-

mozygous TT genotype may be a susceptibility factor of SLE. The TT+CT genotype was a susceptibility factor of LN.

Disclosure of conflict of interest

None.

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