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Original Article

Autoimmune thyroid disease and PTPN22 gene polymorphisms of the correlation analysis

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Abstract: Object: To analyze the relationship between PTPN22 gene polymorphism and autoimmune thyroid disease (AITD), and the relationship between SPTPN22 gene SNP and CTLA-4 gene SNP, in order to investigate the causes leading to AITD. Method: Selected 150 cases with Graves' disease (GD) from February 2014 to March 2015 in our hospital. 135 healthy people were taken as control. A single allele-specific primer - polymerase chain reaction technique (SASP-PCR) was to measure and analyze genotypes and alleles in GD and control groups. Result: In GD group and healthy control group, the distribution frequency of -1123 G>C was consistent with the Hardy-Weinberg genetic equilibrium law, as well as the distribution of CTLA-4 genotype. Comparing the genotype and allele distribution frequency of the two groups, the difference was statistically significant ($P<0.05$), as well as to CTLA-4. The rick of AITD with GG or AG genotype in CTLA-4 and CC genotype in PTPN22 was the 3.23 times of that with AA genotype in CTLA-4 and G genotype in PTPN22. Conclusion PTPN22 1123 G>C SNP genotypes might be related to the occurrence of AITD, and the C allele and the G of CTLA-4 gene could synergistically cause AITD.

Keywords: Autoimmune thyroid disease, PTPN22, gene polymorphism, relevance

Introduction

Autoimmune thyroid disease including hashimoto's thyroiditis, Graves disease and thyroiditis is an immune disease that regards thyroid as target organs [1]. In the pathogenesis of AITD, genetic factors are the important cause of the disease [2], in which, at present, the confirmed genes included CTLA-4 (Cytoxic Tlymphocyto-associated antigen-4) and human leucocyte antigen (HLA) in clinical. Lymphoid specific ammonia acid phosphatase (LyP) coded by PTPN22 gene was a cellular tyrosine phosphatase. More researches in the past showed that there was a correlation between PTPN22 single nucleotide polymorphism of (SNP) and rheumatoid arthritis, diabetes mellitus, system lupus erythematosus disease [3]. Therefore, this study aims to explore the correlation between AITD and SNP PTPN22 gene and analyze the relationship between SPTPN22 gene SNP and CTLA-4 gene SNP.

Materials and methods

General information

150 AITD patients were selected from February 2014 to March 2015 in our hospital, and they were all GD patients (GD group). 135 cases exanimated in our hospital were obtained as healthy control group at the same period. All patients were Han ethnicity; patients of GD group were confirmed by laboratory examination and clinical diagnosis. Age and sex were compared between two groups; there was no statistically significant difference ($P>0.05$). (See **Table 1**).

Method

Genomic DNA extraction of the peripheral blood: The fasting venous blood was collected from all patients by EDTA anticoagulation, and centrifugal column type cell/blood/tissue genomic DNA Extraction Kit (purchased from Beijing days for the Time Inc) was used to extract the genomic DNA from peripheral blood. All operations were carried out strictly in accordance with the kit instructions.

PCR amplification: PCR amplification of exon 14 of PTPN22 gene was as following: primers were designed according to the sequence of PTPN22 gene by ourselves: forward: 5'-TGGGCTCAAGTGATCCTCTCA-3', reverse: 5'-ACTGATAATGTTGC-

Table 1. General data of two groups of patients were compared

Group	Number	Gender [n (%)]		Age (year)
		Male	Famale	
GD group	150	50 (33.33)	100 (66.67)	35.7±13.4
Healthy control group	135	40 (29.63)	95 (70.37)	39.5±14.2
x^2				
P		>0.05		>0.05

TTCAACGGA-3'. The reaction system consisted of 1 μL genomic DNA, 10 μL 2× TaqPCRM asterix, and 1 μL in the forward and reverse primers. PCR was performed under these conditions: Pre denaturation 94°C 3 min; denaturation 94°C 30 s, hybridization 56°C 30 s, and elongation 72°C 30 s for 30 cycles; then elongation 72°C 5 min. The 150 g/L agarose gel electrophoresis was used to identify PCR products.

PCR amplification of PTPN22 promoter was as follow: three primers were designed, a common forward primer: 5'-ACCCTGCATATGTAATGCTGGT-3', two forward primers of 3' specific allele: 5'-CATTGAGAGGTTATGCAAGCTC-3'; 5'-CATTGAGAGGTTATGCAAGCTG-3'. Two pairs of primers were used to amplify the same sample respectively. The reaction system consisted of 1 μL genomic DNA, 10 μL 2× TaqPCR Masterix, and 1 μL in the forward and reverse primers. The elongation step of Primer 1 was 60°C for 30 s, and primer 2 was 62°C for 30 s, the conditions for the rest of the PCR are as above. Agarose The 200 g/L gel electrophoresis was used to identify PCR products.

Amplification of CTLA-4 exon 1: forward primer 5'-GAAGGATGGTGCTTCACAGAT-3'; reverse primer 5'-CTTTGCAGAAGACAGGGATGA-3'. The reaction system consisted of 1 μL genomic DNA, 10 μL 2× TaqPCRM asterix, and 1 μL in the forward and reverse primers. The elongation step of primers was 60°C for 30 s, and the conditions for the rest of the PCR were as above. The 150 g/L agarose gel was used to identify of PCR products.

Enzyme digestion reaction was as follow: all enzyme reaction system included 20 μL and 9 μL the PCR products. PTPN22 exon 14 was treated with 0.5 μL RsaI enzyme overnight at 37°C water bath. CTLA-4 exon 1 was treated with 0.5 μL BbvI enzyme overnight at 65°C water bath. The 250 g/L agarose gel electrophoresis was conducted to identify the enzyme digestion products.

Statistical analysis

The SPSS20.0 statistics software was used to analyze the data. Patient's age and other measurement data was showed as $\bar{x}\pm s$, and t test was conducted. The gene frequencies of each group were expressed as rate, and the χ^2 was used to compare the difference between two groups. And the rank sum test was used to analyze multi-comparison among groups. The $P<0.05$ was showed that the difference is statistically significant.

Results

Comparison of allele type and frequencies of 1123 G>C of PTPN22 gene promoter in two groups

The distribution of polymorphisms genotypes of PTPN22 promoter -1123 G>C was in accordance with Hardy Weinberg equilibrium model in GD group and Healthy control group, there were statistically significant differences in two groups comparison of genotype and allele frequency distribution ($P<0.05$). (See **Table 2**).

Comparison of allele type and frequencies of CTLA-4 gene exon 1+49 A>G of in two groups

The distribution of the polymorphism genotypes of CTLA-4 1+49 A>G was in accordance with Hardy Weinberg equilibrium model, and there were statistically significant differences in two groups compared in genotype and allele frequency distribution ($P<0.05$). (See **Table 3**).

CTLA-4 gene and PTPN22 gene polymorphism and susceptibility of GD

The results showed that the rick of GG or AG genotype of CTLA-4 and CC genotype of PTPN22 was the 3.23 times of AA genotype of CTLA-4 and G genotype of PTPN22. See **Table 4**.

Discussions

PTPN2 gene encodes a lymphoid-specific phosphatase (Lyp) that is important in negative control of T-cell activation and in T-cell develop-

Table 2. Comparison of allele type and frequencies of PTPN22 gene promoter 1123 G>C in the two groups [n (%)]

Group	Number	Distribution frequency of genotype			Distribution frequency of allele	
		GG	GC	CC	G	C
Healthy control group	135	47 (34.81)	70 (51.85)	18 (13.33)	163 (60.37)	107 (39.63)
GD group	150	46 (30.67)	62 (20.67)	42 (28.00)	155 (51.67)	145 (48.33)
x^2			9.569		6.372	
P			<0.05		<0.05	

Table 3. Comparison of allele type and frequencies of CTLA-4 extron 1+49 A>G in the two groups [n (%)]

Group	Number	Distribution frequency of genotype			Distribution frequency of allele	
		AA	AG	GG	A	G
Healthy control group	135	21 (15.56)	64 (47.41)	50 (37.04)	107 (39.63)	163 (60.37)
GD group	150	16 (10.67)	54 (36.00)	80 (53.33)	85 (28.33)	215 (71.67)
x^2			10.192		7.397	
P			<0.05		<0.05	

Table 4. CTLA-4 gene and PTPN22 gene polymorphism and susceptibility of GD

Genotype		GD group	Healthy control group	OR 95% CI
CTLA-4	PTPN22			
AA	GG+GC	13	18	1.00
AA	CC	2	3	1.02 (0.146-7.166)
AG+GG	GG+GC	97	103	1.27 (0.567-2.885)
AG+GG	CC	35	14	3.32 (2.690-8.892)

ment. Lyp is an intracellular protein tyrosine phosphatase with a molecular weight of 110 kDa. The Lyp down-regulates the activation of T cells by binding to the SH3 domain of the Csk kinase, and also dephosphorylates T-cell receptor signaling kinases to inhibit the activation of T cells [4]. In normal human body, regulatory T cells have protective effects on the risk of autoimmune diseases [5]. A research showed that, with the rise of PTPN22 activity, T cell receptor signal transduction would weaken, which contributed to the decreasing of T cell's own regulatory function [6]. Combining the above mechanisms, abnormalities of PTPN22 gene function might lead to autoimmune diseases. PTPN22 gene was currently considered as the most susceptible gene besides CTLA-4 and HLA.

Cao Yali [7] et al reported that the PTPN22 gene 1858T locus had a significant correlation with the occurrence of TID, and this conclusion was confirmed in a number of studies. However, the correlation between the site and diabetes, GD and other diseases were in the United States and Europe and other Caucasian populations, while there was no polymorphisms in Asian populations. Japanese scholar put forward that the PTPN22 gene 1123 G>C locus is correlated with the autoimmune disease and relative frequency higher gene mutations. It showed that the occurrence of type 1 diabetes was related to the PTPN22 gene promoter -1123 G>C, and this correlation reflected in the Korean group, the Japanese population and the Caucasian population [8, 9]. In this study, results showed that frequencies distribution of PTPN22 1123 G>C was in accordance with Hardy Weinberg equilibrium model in GD group and Healthy control group, and there was statistically significant differences in two groups ($P<0.05$). And the distribution frequency of C allele in GD group was significantly higher than healthy control group, which indicated that the PTPN22 1123 G>C SNP genotypes might be related to the occurrence of GD, which was consistent with the literature report [10]. In addition, comparison of allele type and frequencies of CTLA-4 exon 1+49 A>G in the two groups suggested that 1+49 A>G of CTLA-4 gene exon might be related to the occurrence of GD. Results of CTLA-4 gene and PTPN22 gene polymorphisms and susceptibility of GD found that the rick of GG or AG genotype of CTLA-4 and CC genotype of PTPN22 was the 3.23 times of AA genotype of CTLA-4 and G genotype of PTPN22.

The study showed that the risk of occurrence of GD was significantly increased when the CTLA-4 and PTPN22 genes were simultaneously mutated, revealing that the mutation of the two genes might play synergistic roles in the occurrence of autoimmune diseases.

Above all, PTPN22 1123 G>C SNP genotypes might be related to the occurrence of AITD, and the C allele and the G of CTLA-4 gene could synergistically cause AITD.

Disclosure of conflict of interest

None.

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