

Analysis of the Relationship Between IL-10 Gene Polymorphism and Hypertension Susceptibility in Han Nationality in Jiangnan Plain

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Abstract: Objective: To analyze the association between interleukin-10 (IL-10) promoter-1082 and -819 polymorphisms and hypertension in Han nationality in Jiangnan Plain. Methods: A total of 50 Han patients with hypertension in Jiangnan Plain, who were hospitalized in our hospital from March 2018 to April 2018, were selected as the experimental group. 50 healthy Han Chinese people in Jiangnan Plain were selected as the control group. After a series of reactions such as genomic DNA extraction and PCR amplification, polymorphisms of IL-10-819 and -1082 were performed using ABI's BAT3.1 sequencing kit. Analysis and analysis of the risk of developing hypertension at each point. Results: There were three genotypes of C/C, C/T and T/T in the IL-10-819 locus. There was no significant difference between the two groups ($P>0.05$). There were G/G, G/A and A/A genotypes in IL-10-1082 loci in both groups. There was no significant difference between the two groups ($P>0.05$). Logistic regression analysis showed that compared with IL-10-819C/C genotype, the risk of hypertension in the population carrying IL-10-819C/T and T/T genotypes was $OR=1.19$ ($95\%CI=0.71\sim 1.05$), $\chi^2=0.73$, $P>0.05$, $OR=1.05$ ($95\%CI=1.01\sim 1.10$), $\chi^2=0.96$, $P>0.05$, the difference was not statistically significant ($P>0.05$). Compared with IL-10-1082A/A, the risk of developing hypertension in the population expressing IL-10-1082G/A and G/G genotypes was $OR=1.2$ ($95\%CI=0.76\sim 1.12$), $\chi^2=0.704$, $P>0.05$, $OR=1.33$ ($95\%CI=0.77\sim 1.33$), $\chi^2=0.97$, $P>0.05$, the difference was not statistically significant ($P>0.05$). Conclusion: The distribution of genotypes and alleles of IL-10-819 and -1082 loci in Han patients with hypertension and healthy people in Jiangnan Plain is similar, and the genotype mutations of IL-10-819 and -1082 are irrelevant with hypertension.

Keywords: Interleukin-10, Hypertension, Gene polymorphism, Han nationality, Chinese.

1. Introduction

The incidence of hypertension is the result of the participation of multiple mechanisms. In addition to the neuro-endocrine mechanism of genetic, dietary, and mental emotions, genetic and inflammatory mechanisms also play a very important role [1]. In recent years, several studies [2-3] found that hypertension is a chronic low-level inflammatory process, and cytokines play an important role in the process of inflammation. Inflammatory factors are classified into damaging factors and protective factors, IL-10. It is a proven anti-inflammatory cytokine whose physiological function is to regulate the immunosuppressive function of cells. Studies [4] have shown that serum levels of IL-10 are significantly elevated in patients with hypertension, suggesting that IL-10 may be involved in the pathogenesis of hypertension. The biological function and expression of IL-10 are affected by the polymorphism of the promoter region of IL-10 gene, and there is a close relationship between the polymorphic site of promoter region and transcriptional regulator. In this study, we selected the polymorphisms of -819 and -1082 in the IL-10 promoter region of hypertensive patients, and conducted statistical analysis by gene sequencing to demonstrate IL-10 gene polymorphism and hypertension. Relationship.

2. Materials and Methods

General Information 50 cases of Han patients with hypertension in Jiangnan Plain, who were admitted to our hospital from May 2018 to June 2018, were selected as experimental groups(EG), including 23 males and 27 females,

age 29-82 years old, and average (63.24 ± 11.38) years old. In addition, 50 blood samples from healthy people in the same period were collected as a control group(CG), including 26 males and 24 females, age 32-82 years old, and average (62.82 ± 11.41) years old. There were no significant differences between the two groups in age and gender ($p=0.83$, $t=0.211$). Inclusion criteria: (1) hypertension diagnosis according to the 2010 China Guidelines for the Prevention and Treatment of Hypertension [5]; (2) patients agree, signed informed consent; (3) did not use immunosuppressants or immune enhancers; (4) no autoimmune diseases or viral infectious diseases; (5) no obvious damage to the target organs such as heart, eyes, kidney, brain. Exclusion criteria:(1) secondary hypertension; (2) patients do not cooperate; (3) use immunosuppressants or immune enhancers; (4) have autoimmune diseases or viral infectious diseases. The study was approved by the hospital medical ethics committee.

2.1 method

2.1.1 DNA extraction and primer preparation 2ml of fasting blood in the morning, EDTA anticoagulation, DNA extraction using whole blood genome low-dose kit, DNA quality detection and concentration purification. Primers were set according to the IL-10 promoter sequence, and the primer sequence was F: GCAACACTCCTCGCCGC; R: TACCCCGATTTCATTAGGATTCT.

2.1.2 PCR amplification and sequencing

2.1.2.1 Sequencing PCR reaction. The reagent used was ABI's BDT3.1 sequencing kit (BigDye Terminator v3.1), and the sequencing reactions were performed according to the BDT3.1 manual.

2.1.2.2 Purification of sequencing PCR products. Use the

ethanol precipitation method in the BDT3.1 manual, and store it at 4 °C in the dark after drying. As long as the seal is tightly stored for up to one month.

2.1.2.3 Formamide denaturation. 10 ul of formamide was added, and the purified dry powder was sufficiently dissolved, followed by being subjected to a denaturation reaction in a PCR machine.

2.1.2.4 Sample sequencing. The denatured samples were sequenced using 3730XL. Install the capillary according to the instrument manual, perform capillary position correction, manually manually fill the glue and create a running sequencing sequence file. The instrument will automatically fill the gel to the capillary, pre-electrophoresis for 1.2 min at 1.2 kV, auto-inject in the programmed order, pre-electrophoresis (1.2 kV, 20 min), and electrophoresis at 7.5 kV for 2 h. After the electrophoresis is finished, the instrument will automatically clean, fill the gel, enter the next sample, pre-electrophoresis and electrophoresis. The total time of electrophoresis for each sample was 2.5 h. The color

sequencing map is automatically printed after the end of electrophoresis.

2.1.3 Sequence analysis, compare the sequence difference of -819, -1082 between the two groups of IL-10 promoter regions.

2.2 Statistical analysis Count data were analyzed by χ^2 test, and logistic regression was used to analyze the correlation between each genotype and hypertension. The test standard is $\alpha = 0.05$.

3. Experimental Result

3.1 Genotype and frequency distribution: There were three genotypes of C/C, C/T and T/T in IL-10-819 locus in the two groups, and the distribution was not statistically significant ($P>0.05$). There were G/G, G/A and A/A genotypes in IL-10-1082 loci in two groups, and the distribution was not statistically significant ($P>0.05$). See Table 1. figure 1.

Table 1. Genotype and frequency distribution in the groups

Groups	Numbers of cases	IL-10-819			Allele		IL-10-1082			Allele	
		C/C	C/T	T/T	C	T	G/G	G/A	A/A	G	A
CG	50	14(28%)	19(38%)	17(34%)	47(47%)	53(53%)	14(28%)	20(40%)	16(32%)	48(48%)	52(52%)
EG	50	13(26%)	21(42%)	16(32%)	47(47%)	53(53%)	15(30%)	21(42%)	14(28%)	51(51%)	49(49%)

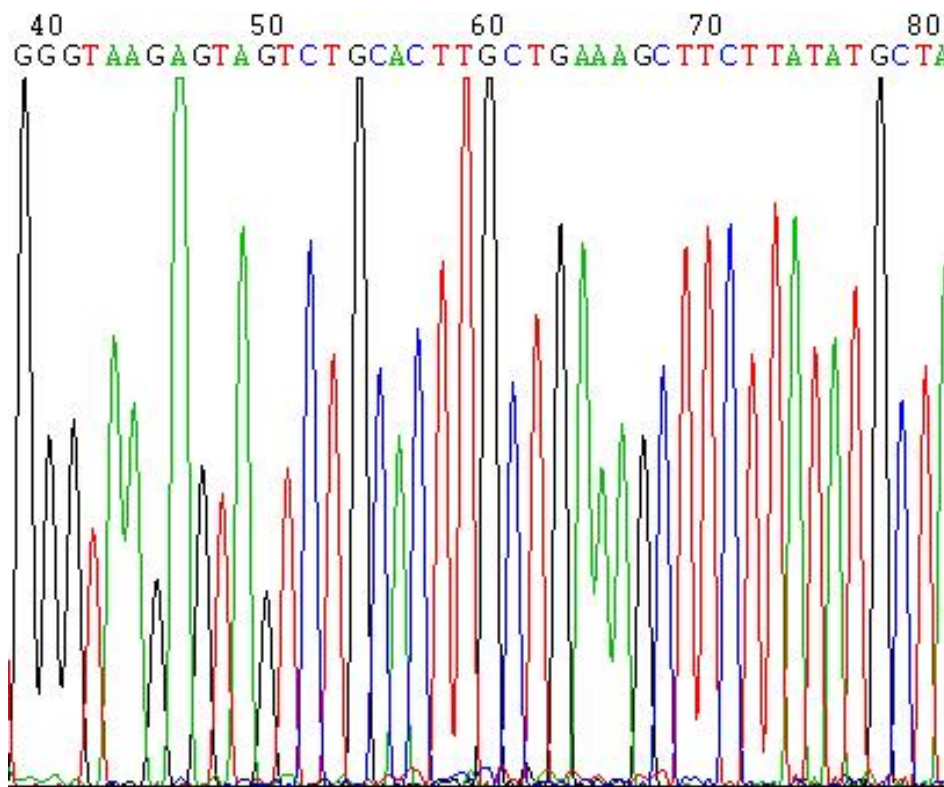


Figure 1. Genotype distribution

3.2 Genotype frequency Hardy-Weinberg equilibrium test: The actual value obtained in this study is compared with the predicted value, and the χ^2 test conforms to the Hardy-

Weinberg equilibrium ($P>0.05$). It was confirmed that the population of this study accorded with the laws of genetics and was representative. See Table 2 and 3.

Table 2. Frequency balance test of IL-10-819 loci in two groups

Groups	Numbers of cases	C/C	C/T	T/T	χ^2	P value
The control group	50					
The actual value		14	19	17	1.44	>0.05
The predicted value		11	25	14		
The text group	50					
The actual value		13	21	16	0.99	>0.05
The predicted value		11	25	14		

Table 3. Frequency balance test of IL-10-1082 loci in two groups

Groups	Numbers of cases	G/G	G/A	A/A	χ^2	P value
The control group	50					
The actual value		14	20	16	1.19	>0.05
The predicted value		12	25	13		
The text group	50					
The actual value		15	21	14	0.96	>0.05
The predicted value		13	25	12		

3.3 Correlation analysis between genetic loci and breast cancer genetic susceptibility. The results obtained in this study were analyzed by logistic regression analysis to verify

the correlation between IL-10-819 and IL-10-1082 points and genetic susceptibility to hypertension. See Table 4 and 5.

Table 4. Risk analysis of genetic predisposition of IL-10-819 locus polymorphism in hypertension

SNP	OR	95%CI	χ^2	P value
C/C	1			
C/T	1.19	0.71—1.05	0.73	>0.05
T/T	1.05	1.01—1.10	0.96	>0.05

Table 5. Risk analysis of genetic predisposition of IL-10-1082 locus polymorphism in hypertension

SNP	OR	95%CI	χ^2	P value
A/A	1			
G/A	1.2	0.76-1.12	0.704	>0.05
G/G	1.33	0.77-1.33	0.97	>0.05

4. Discuss

There are many factors involved in the occurrence and development of hypertension. In addition to factors such as renin angiotensin system, sympathetic activation and high salt diet, inflammation is also involved in the pathological mechanism of hypertension, and cytokines are an important process of inflammation. Also involved in the pathogenesis and development of hypertension. As an important anti-inflammatory cytokine, IL-10 is significantly elevated in the serum of hypertensive patients [4], which is also bound to participate in the pathogenesis of hypertension. IL-10 belongs to the interferon family of cytokine synthesis inhibitors [6], which can act on mononuclear-macrophages, inhibit the release of immune mediators, and reduce TNF- α , IL-1, IL-6, IL-8, Production and secretion of factors such as IL-12 [7]. IL-10 also has the ability to inhibit the proliferation and migration of inflammatory cells. In addition, IL-10 can also play an important role in the regulation of vascular endothelial cells in the development and progression of hypertension [8]. However, there are transcription factors in the promoter region of IL-10, which affects the expression of its level and further affects its biological activity. Therefore, the study of gene polymorphism in promoter region is a hot topic in current research. Therefore, we also selected the genetic polymorphisms of the IL-10 promoter region at -819 and -1082 in hypertensive patients, and conducted statistical analysis by gene sequencing to demonstrate IL-10 gene polymorphism and hypertension. The relationship provides

new ideas for the prevention and treatment of hypertension.

In the study, we found that there were three genotypes of C/C, C/T and T/T in the IL-10-819 locus in the experimental group and the control group, and G/G and G were present in the IL-10-1082 locus. There was no significant difference in the /A and A/A genotypes between the two groups ($P>0.05$). Logistic regression analysis showed that compared with IL-10-819C/C genotype, the risk of hypertension in the population carrying IL-10-819C/T and T/T genotypes was OR = 1.19 (95%CI = 0.71~1.05), $\chi^2=0.73$, $P>0.05$, OR = 1.05 (95%CI = 1.01~1.10), $\chi^2=0.96$, $P>0.05$, the difference was not statistically significant ($P>0.05$). Compared with IL-10-1082A/A, the risk of developing hypertension in the population expressing IL-10-1082G/A and G/G genotypes was OR = 1.2 (95%CI = 0.76~1.12), $\chi^2=0.704$, $P>0.737$, OR = 1.33 (95% CI = 0.77~1.33), $\chi^2=0.97$, $P>0.05$, the difference was not statistically significant ($P>0.05$). This further revealed that the IL-10-819 and -1082 genotype mutations in the Han population of Jiangnan Plain were not associated with the onset of hypertension. The occurrence of hypertension is the result of the interaction of multiple environmental factors, multiple pathological processes, and multiple genetic factors. Relative to the susceptibility factors of hypertension, the presence of this polymorphism site interacts with other risk factors and plays a role in the occurrence and development of the disease. The single factor occurs in the Han nationality patients in Jiangnan Plain. It does not work in isolation with development.

Due to the short time of this study, the sample size collected

is small, and the genetic polymorphism is greatly affected by the environment and ethnicity. Therefore, the refinement of the population classification will be further studied.

References

- [1] Yufen Gao, Jingkai Chan and Gangjun Chung. Changes of microalbuminuria and inflammatory factors in prehypertensive population[J]. Chongqing Medical Journal, 2014, 43(21): 2788-2791.
- [2] Anand R, Nair, Philip J. Angiotensin II-induced hypertensive renal inflammation is mediated through HMGB1-TLR4 signaling in rat tubulo-epithelial cells[J]. Experimental Cell Research, 2015, 335(2): 238-247.
- [3] Zhaopeng Chou, Zujian Hui and Xiaohua Day. Relationship between essential hypertension and inflammatory factors and intervention of traditional Chinese medicine[J]. Journal of Traditional Chinese Medicine and Clinical Medicine, 2015, 27(3): 307-310.
- [4] Hua Chang, Lixiang Liu and Zhi Liu. Changes and significance of serum IL-6, IL-10 and TNF- α levels in patients with diabetes mellitus complicated with hypertension[J]. Shandong Medical Journal, 2015, 55(19): 79-80.
- [5] China Hypertension Prevention and Treatment Guidelines Revision Committee. China Guidelines for Prevention and Treatment of Hypertension 2010[J]. Chinese Journal of Hypertension, 2011; 19(8): 701-743.
- [6] Ruicheng Hu, Yongjian Xu, Zhenxiang Chang and et. Relationship between interleukin-10 gene promoter polymorphism and susceptibility to chronic obstructive pulmonary disease [J]. Chinese Journal of Medical Genetics, 2003; 20: 503-507.
- [7] Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease [J]. Nature Medicine, 2012, 18(3): 363-374.
- [8] Zhaohui Chiang, Jianjun Wong and Wenmin Chan. Determination and significance of TNF- α and NF- κ B in serum of patients with essential hypertension[J]. Medical Clinical Research, 2014, 31(3): 527-530.