Genetic variants in the tumor necrosis factor receptor 2 gene in patients with Systemic lupus erythematosus*

YIN Zhi - hua, YE Zhi - zhong, ZHUANG Jun - han, GAN Da - qiang, WANG Xun, LUO Xiu - xia

(*Xiangmihu Rheumatology Branch, Fourth People's Hospital of Shenzhen, Shenzhen Rheumatology Institute of Guangdong Medical College, Shenzhen* 518040, *Guangdong Province, China*)

ABSTRACT: In this paper, we designed to investigate the frequencies of tumor necrosis factor receptor 2 (TNFR2) polymorphisms at nt587 and nt694 in south Chinese SLE patients and healthy individuals and explore whether genetic variants in TNFR2 gene is involved in the pathogenesis of SLE. The results showed that the nt587G allele frequency was 21.1% in the 128 SLE patients and the allele frequency was 13.0% in the 135 healthy individuals, the former was significantly higher than the latter in the allele frequency ($P < 0.05$). People with the nt587 G variant showed high risk to SLE. The frequency of nt694 was slightly but not statistically significantly increased in SLE patients compared with healthy controls(16.0% versus 11.9%, $P = 0.149$). These results indicate that the polymorphism at nt587 of TNFR2 is associated with the south Chinese SLE patients. The polymorphism at nt694 is not associated with SLE.

Key words: Systemic lupus erythematosus; Tumor necrosis factor receptor 2; Polymorphism

Introduction

Systemic lupus erythematosus (SLE) is a polygenic autoimmune disorder affecting multiple organ systems. Multiple genetic as well as environmental factors are considered to be involved in the development of SLE.

Tumor necrosis factor receptor 2 (TNFR2) had been proved to mediate membrane TNF - α to augment the immune response of T cells and B cells in vitro. Therefore TNFR2 was considered to be a strong candidate for a susceptibility gene to SLE. Komata et al[1] demonstrated a significant association of nucleotide (nt) 587 * T→G in TNFR2, also as codon 196 Met→Arg, with SLE in the Japanese. However, a subsequent study did not show any association between this site and SLE in either Spanish, UK, or Korean populations[2,3]. Since thisdiscrepancy might be due to the genetic heterogeneity among different ethnic groups, it is necessary to confirm the association of nt 587 * T →G with SLE in the Chinese population. A recent report[4] has revealed that a new polymorphism site nt 694 * G→A is also associated with non - conserved amino acid substitution: Glutamic acid (Glu) → lysine (lys).

No one has studied the association of nt 694 * G→A with SLE. In the present study, in order to find the association of TNFR2 and SLE in Chinese population, we analysed the polymorphisms at nt587 and nt694 of TNFR2 in the Chinese SLE patients and healthy controls.

1 Materials and methods

1.1 Materials

One hundred twenty - eight Chinese patients with SLE (114 females, 14 males), who were being hospitalized in the rheumatology department of Shenzhen Xiangmihu Rheumatology Hospital during 2003 to 2005 , were studied. All patients accorded the American College of Rheumatology 1982 revised criteria for SLE. The average age of the patients was 33.6 ± 14.8 years (range 14 - 65 years). One hundred thirty - five healthy Chinese individuals were also enrolled as controls, consisting of 110 females and 25 males aged from 8 to 75 years. Their average age was 40.3 ± 17.8 years. Informed consent was obtained from all patients and healthy individuals.

1.2 DNA extract

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) as previously described.

1.3 PCR - SSP analysis

The primers for sequence - specific primer PCR for the two sites of nt587 and nt694 were synthesized based on the genomic DNA sequence of the TNFR2 gene (Table 1), PCR was performed by using 50 ng genomic DNA as template, 0.5 μM of each primer, 0.2 mM of each dNTP, and 0.25 units Taq polymerase (Bolight, Shanghai, China) in a total reaction of 20 μl. Reactions were conducted for 30 cycles consisting of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C using a PTC - 100 thermal cycler (MJ Research, USA). PCR products were subjected to electrophoresis on 1.5% agarose gels under a constant voltage of 80V for twenty minutes and then examined under UV illumination and documented by photography.

Table 1 Primers for analysis of the tumor necrosis factor receptor 2 genome

Name	Nucleotide sequence (5' - 3')
TNFR2 - 587F	CTCCTATCCTGCCTGCT
TNFR2 - 587RT	GACGTGCAGACTGCATCCA
TNFR2 - 587RG	GACGTGCAGACTGCATCCC
TNFR2 - 694F	CTCCTATCCTGCCTGCT
TNFR2 - 694RG	CTTGCAGCAGTGCTGGGTTC
TNFR2 - 694RA	CTTGCAGCAGTGCTGGGTTT

F: forward; R: reverse

1.4 Statistical analysis

TNFR2 allele frequencies were determined by direct counting. Allelic frequency was calculated with the following equation: allelic

* Supported by: Medical Key Subject Foundation of Shenzhen City, No.2005c10

Author: Yin Zhi - hua, (1973 -), female, Doctor, attending physician, major in the study of the molecular mechanism of SLE

Correspondence to: Yin Zhi - hua, E - mail: yzh2000@163.com

Received: Jul 20, 2006 Accepted: Aug 31, 2006

frequency = n/2N (n represents the number of this allele, and N represents the total number of subjects. Allelic frequencies were compared using the Chi - square test. A P value less than 0.05 was considered statistically significant. The strength of an association was expressed using odds ratio (OR) with 95% confidence interval.

2 Results

2.1 Association of TNFR2 nt 587 * T→G allele with susceptibility to Chinese SLE

The allele frequency of G at nt 587 (196R) was significantly increased in SLE patients (21.1%) compared with healthy controls (13.0%) as shown in Table 2(odds ratio 1.79, P = 0.013) and Figure 1. Of the 128 SLE patients,3(2.3%) were homozygous and 48(37.5%) were heterozygous, while 33 of 135 controls(24.5%) were heterozygous and 1 was homozygote. The genotype 587 G/G was also significantly associated with SLE (P = 0.033).

Table 2 Comparison of TNFR2 nt 587 polymorphism between all patients and controls

	All SLE(%) (n = 128)	All controls(%) (n = 135)	x^2	P[a]	Odds ratio (95% CI[b])
Allele positivity					
587G	54(21.1)	35(13.0)	6.18	0.013	1.79(1.13 - 2.86)
587T	202(78.9)	235(87.0)			
Genotype frequency					
587G/G	3(2.3)	1(0.7)	6.83	0.033	
587T/G	48(37.5)	33(24.5)			
587T/T	77(60.2)	101(74.8)			

[a]Two - sided P values were calculated by x^2 test from 2 × 2(for comparison of allele positivity)and 2 × 3 (for comparison of genotype frequence) contingency tables

[b]CI = confidence interval

2.2 Lack of association between TNFR2 nt 694 polymorphism and Chinese SLE

The allele frequency of A at nt 694 was 16.0% in SLE patients and 11.9% in healthy controls as shown in Table 3(P = 0.167). The genotype 694A/A was also not statistically significantly increased in SLE patients compared with healthy controls(P = 0.304).

Table 3 Comparison of TNFR2 nt694 polymorphism between all patients and controls

	All SLE(%) (n = 128)	All controls(%) (n = 135)	x^2	P[a]	Odds ratio (95% CI[b])
Allele positivity					
694A	41(16.0)	32(11.9)	1.91	0.167	1.42(0.86 - 2.33)
694G	215(84.0)	238(88.1)			
Genotype frequency					
694A/A	2(1.6)	2(1.5)	2.38	0.304	
694A/G	37(28.9)	28(20.7)			
694G/G	89(69.5)	105(77.8)			

[a]Two - sided P values were calculated by x^2 test from 2 × 2(for comparison of allele positivity)and 2 × 3 (for comparison of genotype frequence) contingency tables

[b]CI = confidence interval

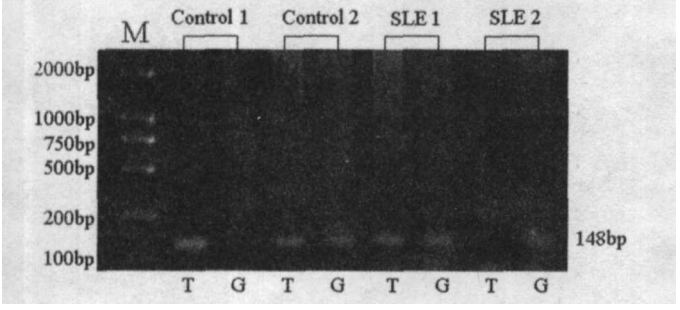

Fig.1 The results of nt 587 representative genotypes of TNFR2.
control 1: T/T allele; control 2 : T/G allele;
SLE 1: T/G allele; SLE 2: G/G allele.

3 Discussion

In the present study, we confirmed the polymorphism of nt 587 within TNFR2, which was comparable with earlier findings[5] in the Japanese population(20.5% versus 12.6%). It can be concluded that, like Japanese, the 587G allele is involved in susceptibility to SLE in south - China. Moreover the presence of only one 587G (196R) allele was shown to be sufficient for the susceptibility to SLE, suggesting that this amino acid substitution has either gain - of - function or dominant negative effect with a low penetrance.

There are two distinct receptors for TNF - α, TNFR1 and TNFR2. Both receptors have many similar functions, but some functions seem to be unique to TNFR2. For example, TNFR2 was shown to be increased in the microvascular endothelial cells and to mediate upregulation of ICAM - 1 when stimulated by membrane - bound TNF[6]. If signals transmitted from TNFR2 bearing 587G are stronger than those from 587T allele, it is reasonable to assume that individuals with this allele may be more prone to small vessel vasculitis, which constitutes one of the main features of SLE. Some experiments have confirmed the assumption. By functional study, Morita et al[5] demonstrated that

587G TNFR2 could increase more IL-6 production and induce more cytotoxic activity than 587T TNFR2. In a recent study[7], it was found that the Met-196→Arg variation of human TNFR2 affected TNF-α-induced apoptosis by impaired NF-κB signalling and target gene expression. This result could be served as an explanation for the association of the TNFR2 587G variant with an increased susceptibility for SLE.

4 Conclusion

In conclusion, we analysed the polymorphisms of two sites of the TNFR2 gene using PCR-SSP analysis.

We showed an association of the TNFR2 polymorphism at nt 587, but not at nt 694 with SLE in South-China, which was accordant with that in the Japanese. Further study on the association of this TNFR2 polymorphism with SLE in other ethnic groups and with other autoimmune diseases is warranted.

References

[1] Komata T, Tsuchiya N, Matsushita M, et al. Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus[J]. Tissue Antigens, 1999,53(6):527-533

[2] Al-Ausari AS, Ollier WER, Villarreal J, et al. Tumor necrosis factor receptor Ⅱ (TNFR Ⅱ) exon 6 polymorphism in systemic lupus erythematosus[J]. Tissue Antigens, 2000,55(1):97-99

[3] Lee EB, Yoo JE, Lee JY, et al. Tumor necrosis factor receptor 2 polymorphism in systemic lupus erythematosus: no association with disease [J]. Hum Immunol, 2001,2(10):1148-1152

[4] Ehling R, Gassner CH, Lutterotti A, et al. Genetic variants in the tumor necrosis factor receptor Ⅱ gene in patients with multiple sclerosis[J]. Tissue Antigens, 2004,63(1):28-33

[5] Morita C, Horiuchi T, Tsukamoto H, et al. Association of tumor necrosis factor receptor type Ⅱ polymorphism 196R with systemic lupus erythematosus in the Japanese[J]. Arthritis Rheum, 2001,44(12):2819-2827

[6] Canault M, Peiretti F, Mueller C, et al. Proinflammatory properties of murine aortic endothelial cells exclusively expressing a non cleavable form of TNFalpha. Effect on tumor necrosis factor alpha receptor type 2[J]. Thromb Haemost. 2004,92(6):1428-1437

[7] Till A, Rosenstiel P, Krippner-Heidenreich A, et al. The Met-196→Arg variation of human TNFR2 affected TNF-α-induced apoptosis by impaired NF-κB signalling and target gene expression[J]. J Bio Chem, 2005,280(7):5994-6004

TNFR2基因多态性与SLE的相关性研究*

尹志华 叶志中 庄俊汉 甘达强 汪 迅 罗秀霞

(广东省深圳市第四人民医院香蜜湖风湿病分院、广东医学院深圳风湿病研究所 深圳 518040)

摘要:本研究通过调查中国南方SLE人群和健康对照人群中TNFR2基因两个位点(nt587,nt694)的多态性频率,探讨TNFR2基因多态性是否与中国汉族SLE人群的易感性相关。结果发现128例SLE中,nt587G的等位基因频率为54个(21.1%);而135例健康人群中nt587G的频率为35个(13.0%);SLE组明显高于健康对照人群(P<0.05),携带nt587G的个体SLE发病危险性大。同时128例SLE中,nt694 A的等位基因频率为41个(16.0%);而健康人群中nt694 A的频率为32个(11.9%);两组比较无显著差异(P=0.149)。提示TNFR2基因nt587的多态性与中国南方SLE人群相关,可能通过影响TNFR2的表达而参与SLE的发病,而nt694(G-A)的多态性与中国南方SLE人群不相关。

关键词:系统性红斑狼疮;肿瘤坏死因子受体2;多态性

* 基金项目:深圳市医学重点学科建设资助项目(2005c10)

作者简介:尹志华,(1973-),女,博士,主治医师,主要研究方向:系统性红斑狼疮的发病机制研究

通讯作者:尹志华,E-mail:yzh2000@163.com

(收稿日期:2006-07-20 接受日期:2006-08-31)