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Original Article

TNF-α and *IL10* polymorphisms interaction increases the risk of ankylosing spondylitis in Chinese Han population

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Abstract: Aims: The target of this article was to reveal the role of tumor necrosis factors α (TNF-α) and Interleukin-10 (IL10) gene polymorphisms in ankylosing spondylitis (AS) development and explore the interaction between these two gene polymorphisms. Methods: The genotyping of gene polymorphims was conducted using ABI Taqman assay method in 84 AS patients and 92 healthy people. Hardy-Weinberg equilibrium (HWE) was checked in the control group and the genotypes and alleles difference were compared with χ^2 test. Odds ratio (OR) with 95% confidence interval (CI) was calculated to identify the strength of association between gene polymorphism and disease. Meanwhile, multifactor dimensionality reduction (MDR) method was used to analysis the interaction between gene polymorphisms. Results: The genotypes CG+CC of the minor allele in IL10 rs1878672 in cases was obviously higher frequency than the controls (*P*=0.03) and the minor allele C was also associated with the increased risk of AS, compared with G allele (OR=2.05, 95% CI=1.08-3.89). Rs3024490 in IL10 also showed a significant correlation to the onset risk of AS (GG vs. TT: OR=3.03, 95% CI=1.04-8.87; G vs. T: OR=1.70, 95% CI=1.08-2.68). What's more, there was the interaction between TNF-α rs3093662 and IL10 rs3021094, rs3024490 polymorphisms in AS. Conclusions: IL10 rs1878672 and rs3024490 polymorphisms obviously increase the susceptibility to AS, but not TNF-α rs3093662. Both IL10 and TNF-α polymorphisms may affect the onset of AS.

Keywords: *IL10*, *TNF-α*, polymorphism, ankylosing spondylitis, interaction

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder which mainly invades axial skeleton and characterized by sacroilioarthritis and involving in eye, lung, kidney and cardiovascular system in a variable extent [1, 2]. This disease strikes the adults in 20-60 years old mostly with the high incidence, which gives rise to heavy socio-economic burden for patients' family [3]. Human leucocyte antigen (HLA)-B27 is the first certain molecule proved to be strongly associated with AS [4, 5]. Unfortunately, HLA-2B only accounts for the part risk of AS, what's more, a number of previous researchers have found that some other genes, including *ANTXR2*, *KIF21B*, *ERAP1*, *TNF-α* and *IL10* significantly are correlated with AS susceptibility [6-10].

Tumor necrosis factors α (TNF-α), belonging to TNF family, is a kind of cytokine caused cell death. It is encoded by *TNF-α* gene located on chromosome 6p21.3, including 4 exons and 3 introns [11]. Interleukin-10 (IL10) is an anti-inflammatory cytokine and encoded by *IL10* gene in chromosome 1q31-32, containing 5 exons [12]. Many single nucleotide polymorphisms (SNPs) have been identified in *TNF-α* and *IL10* gene, in the meanwhile, various studies have found that some SNPs can affect the transcription level of *TNF-α* and *IL10* [13, 14]. They may be associated with infectious, autoimmune diseases and cancers and interfere into the generation, development of diseases. The research showed that the expression level of TNF-α mRNA in AS patients was higher than healthy people and the therapy with anti-TNF-α antibody and TNF-α receptor antagonist for AS

Table 1. Characteristics of subjects in the case and control groups

Index		Cases/84	Controls/92	*P*
Age ($\bar{X}$±s)	Mean age	32.52±8.74	33.29±8.66	0.56
Sex, n(%)	Female	32 (38.10)	39 (42.39)	0.56
	Male	52 (61.90)	53 (57.61)	

patients could improve the symptoms [15]. Meanwhile, the levels of IL10 were significantly higher in AS patients than the healthy people and the haplotype of IL10 increased the susceptibility to AS in Chinese population [16]. Hence, the two genes served as candidate susceptibility genes for AS.

However, most of the early researches focused on the role of single gene polymorphisms in AS. The aim of the study was to investigate the association of *TNF-α* rs3093662 and *IL10* rs1878672, rs3021094, rs3024490 SNPs and their interactions with AS susceptibility and hoped to gain more clues for explaining the etiology of AS.

Materials and methods

Subjects

This study was conducted with a case-control design. 84 AS patients with positive HLA-B27 diagnosed by the pathology department in Provincial Hospital affiliated to Shandong University (Jinan, Shandong Province) were selected as the case group from 4, 2012 to 11, 2013. The cases age range was 15-42 and included 32 females and 52 males. Besides, the patients were excluded if they were simultaneously subject to tumors, cardiovascular system disease and the other inflammatory diseases or once had the relative disease history. In the control group, 92 healthy persons were enrolled, who experienced the physical examination in the same hospital with the cases at the same time and their age and gender were frequency-matched with the cases. All subjects were Han population and meanwhile they had no relationship by blood. The study was approved by the Research Ethics Committee of above hospital and written consents were obtained from all participants in our study.

Genotyping

Blood samples were collected from each subject and stored at -80°C refrigerator for next step. And then blood DNA extraction was conducted by the method of the conventional chloroform isoamyl alcohol extraction.

The genotyping of rs3093662 in *TNF-α* and rs3024490, rs1878672 and rs3021094 in *IL10* was conducted utilizing an ABI Taqman assay on a 7900 system (Applied Biosystems, California), according to the manufacturer's instructions [10]. All samples were measured in 3 replicates.

Statistical analysis

SPSS 18.0 software was used in statistical analysis. The genotypes distributions of *TNF-α* and *IL10* SNPs were verified it they conformed to Hardy-Weinberg equilibrium (HWE) among the controls. The differences were judged based on t-test by age and χ^2 test by the other indexes. The data were represented with n (%) or $\bar{X}$±s and odds ratio (OR) with corresponding 95% confidence interval (95% CI) was used to reveal the association intensity of gene polymorphism with disease susceptibility. $P<0.05$ was considered as the statistically significant meaning. At last, the interaction among gene polymorphisms was analyzed by the method of multifactor dimensionality reduction (MDR) in AS.

Results

Demographic and clinical data of the cases and controls

In this article, 176 subjects were enrolled including 84 AS patients and 92 healthy people. The males accounted for 61.90% and 57.61% in two groups respectively and there was no significant difference between the two groups ($P=0.56$). The average age of participants in cases was 32.52±8.74 and the control group was a little higher (33.29±8.66), but the difference was not significant ($P=0.56$) (**Table 1**).

Effects of SNPs in TNF-α and IL10 genes on AS susceptibility

HWE was tested before the study, the results indicated that the genotypes distributions of *TNF-α* and *IL10* SNPs in the control group conformed to HWE and the population possessed a representativeness ($P>0.05$).

Any genotypes and alleles of *TNF-α* rs3093662 polymorphism had no significant difference in

Table 2. Relationship of the genotypes and alleles distribution *TNF-α* and *IL10* polymorphisms with AS susceptibility

Polymorphism	Genotypes/ alleles	Case/n (%)	Control/n (%)	OR (95% CI)	*P*
TNF-α					
rs3093662	AA	18 (21.43)	25 (27.17)	1.00	
	AG	41 (48.81)	47 (51.09)	1.21 (0.58-2.53)	0.61
	GG	25 (29.76)	20 (21.74)	1.74 (0.75-1.04)	0.20
	A	77 (45.83)	97 (52.72)	1.00	
	G	91 (54.17)	87 (47.28)	1.32 (0.87-2.00)	0.20
IL10					
rs1878672	GG	60 (71.43)	78 (84.78)	1.00	
	CG+CC	24 (28.57)	14 (15.22)	2.23 (1.06-4.67)	0.03
	G	139 (82.74)	167 (90.76)	1.00	
	C	29 (17.26)	17 (9.24)	2.05 (1.08-3.89)	0.03
rs3021094	CC	34 (40.48)	33 (35.87)	1.00	
	CA	35 (41.67)	41 (44.56)	0.83 (0.43-1.60)	0.58
	AA	15 (17.86)	18 (19.57)	0.81 (0.35-1.87)	0.62
	C	103 (61.31)	107 (58.15)	1.00	
	A	65 (38.69)	77 (41.85)	0.88 (0.57-1.34)	0.55
rs3024490	TT	33 (39.29)	50 (54.35)	1.00	
	TG	39 (46.43)	36 (39.13)	1.64 (0.87-3.09)	0.12
	GG	12 (14.28)	6 (6.52)	3.03 (1.04-8.87)	0.04
	T	105 (62.50)	136 (73.91)	1.00	
	G	63 (37.50)	48 (26.09)	1.70 (1.08-2.68)	0.02

Table 3. The best models of *TNF-α* and *IL10* gene polymorphisms interaction

Model	Gene	Locus	Cross-validation consistency	Testing accuracy (%)	OR (95% CI)	*P*
2	*IL10*	rs1878672	8/10	53.40	2.62 (1.34-5.13)	0.62
		rs3024490				
3	*TNF-α*	rs3093662	10/10	56.80	4.51 (2.28-8.95)	0.01
	IL10	rs3021094				
		rs3024490				
4	*TNF-α*	rs3093662	10/10	54.71	6.62 (3.26-13.45)	0.17
	IL10	rs1878672				
		rs3021094				
		rs3024490				

these two groups (*P*>0.05) and it was not an independent risk factor for AS. As was shown in **Table 2**, we also gained the similar result in *IL10* rs3021094 polymorphism (*P*=0.58, 0.62, 0.55, respectively). Differently, the mutant genotypes CG+GG of *IL10* rs1878672 polymorphism had a higher frequency in cases than that of the controls (28.57% & 15.22%) and it significantly increased the susceptibility to AS (OR=2.23, 95% CI=1.06-4.67, *P*=0.03). So was the mutant allele C, compared with allele G (OR =2.05, 95% CI=1.08-3.89, *P*=0.03). In the analysis, we also found that GG genotype and G allele of rs302-4490 might increase the risk for AS (GG vs. TT: OR =3.03, 95% CI=1.04-8.87; G vs. T: OR=1.70, 95% CI=1.08-2.68).

The interaction analysis of TNF-α and IL10 SNPs based on AS

The interaction analysis results of *TNF-α* and *IL10* SNPs were displayed in **Table 3**. In the best model of two sites, three sites and four sites, only 3 sites (rs-3093662, rs3021094, rs3024490) had a statistically significant meaning (*P*=0.01) and was the best model in overall interaction analysis. Its testing accuracy was 56.80% and meanwhile, the cross-validation consistency was very good (10/10) in this model. Therefore, there existed the interaction between *TNF-α* and *IL10* SNPs and they played the role in the development of AS synergistically.

Discussion

TNF served as a multifunctional pro-inflammatory cytokine, which not only is it associated with various physiological processes but also involves in several pathological processes, such as apoptosis, proliferation, inflammation and immunoreguation [17-19]. The up-regulated expression of *TNF-α* is associated with various illnesses involved inflammatory, autoimmune system disorders and tumors. The previ-

ous studies showed that the patients with active AS showed the increased levels of TNF-α in sacroiliac joint, which suggested an important role of *TNF-α* in AS occurrence [20]. There is no doubt that some functional genetic variants of *TNF-α* can affect its expression in transcription and translation level [21], so the influence of polymorphsims in *TNF-α* on the diseases drown scientists' attention widely. Among them, -308G/A polymorphism in *TNF-α* promoter region has been reported to involve in various diseases, including AS in multiple populations. Manolova et al. found that this SNP played a role in AS occurrence and determining the age of onset, disease severity and the outcome of treatment in AS patients based on the Bulgarian population [22]. Ji et al. show that -308G/A polymorphism in *TNF-α* may be a weak indicator to identify the active state of AS in Chinese population [23]. In this article, *TNF-α* rs3093662 was explored the association with AS, but the relationship wasn't found in our population.

IL10, as an important anti-inflammatory factor can inhibits the synthesis of pro-inflammatory cytokines IL-4, IL-5 and decrease the level of immunoglobulin E (IgE) and the number of eosinophil granulocyte (EOS) in blood. IL10 can suppress not only the generation of Th1, Th2 cytokines but the cloning and proliferation of T-cells induced by antigen, and the expression of IL10 in patients with allergic disease is lower than healthy controls, which considers that IL10 may regulate the function of others cytokines and play the immunomodulatory role in the pathologic process of allergic disease, according to the study of Kawamoto et al. [24]. IL10 exists a mass of SNPs [25], such as -1082G/A, -819C/T, -592C/A, -627C/A polymorphisms, which may affect the transcription activity of *IL10* and its expression in plasma. What's more, *IL10* polymorphism has been found to contribute to the susceptibility to AS in a Chinese population [8]. At the same time, *IL10* rs1878672, rs3024490 polymorphisms were also identified the significant correlation to AS risk and might affect the AS occurrence through regulating the expression of *IL10* in plasma.

In the meanwhile, because IL10 serves as an effective inhibitor of TNF, the relative researches has showed that the differences in the regulation of IL10 and TNF-α play critical roles in the abnormal inflammatory responses [26]. We all know that in early, researches almost involved in the association between polymorphisms of single gene and AS susceptibility. Increasingly, some scientists attempted to study the association of multiple gene polymorphisms interaction and AS susceptibility. Zhang et al. studied the association of *HLA-B27* and *ERAP1* with AS susceptibility and concluded that the interaction of *HLA-B27* and *ERAP1* was likely to increase the risk for AS [27]. Similarly, the relationship of *HLA-B27* subtypes and *TNF-α* polymorphism with AS in Iranian population were investigated and the results showed that there was no interaction between the -238A/G polymorphism of *TNF-α* and *HLA-B27* subtypes to AS susceptibility [28]. In our study, the interaction of *IL10* and *TNF-α* polymorphisms was analyzed based on AS and the conclusion was that *TNF-α* rs3093662 and *IL10* rs3021094, rs3024490 SNPs existed the interaction and affected the generation and progression of AS synergistically. Therefore, it suggests that the genetic variants influence the expression level of gene and regulate the ratio of TNF-α and IL10 in plasma to involve in relative diseases.

In conclusion, AS is a complicated and multifactorial disease characterized by familial inheritance, so genetic variant of relative gene is an important direction to explain the etiology and pathology of AS. Rs3093662 in *TNF-α* and rs1878672, 3024490 in *IL10* are revealed to significantly increase the risk of AS, meanwhile, there was the interaction between *TNF-α* rs3093662 and *IL10* rs3021094, rs3024490 SNPs in AS.

Disclosure of conflict of interest

None.

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References

[1] Jenkinson TR, Mallorie PA, Whitelock HC, Kennedy LG, Garrett SL and Calin A. Defining spinal mobility in ankylosing spondylitis (AS). The Bath AS Metrology Index. J Rheumatol 1994; 21: 1694-1698.

[2] Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, Taylor A, Calin A and Wordsworth P. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997; 40: 1823-1828.

[3] Bakland G, Gran JT, Becker-Merok A, Nordvag BY and Nossent JC. Work disability in patients with ankylosing spondylitis in Norway. J Rheumatol 2011; 38: 479-484.

[4] de Bruyere M and Nagent de Deuxchaisnes C. Segregation of HL-A27 and ankylosing spondylitis in an informative kindred. Tissue Antigens 1976; 7: 15-22.

[5] Hanova P, Pavelka K, Holcatova I and Pikhart H. Incidence and prevalence of psoriatic arthritis, ankylosing spondylitis, and reactive arthritis in the first descriptive population-based study in the Czech Republic. Scand J Rheumatol 2010; 39: 310-317.

[6] Karaderi T, Keidel SM, Pointon JJ, Appleton LH, Brown MA, Evans DM and Wordsworth BP. Ankylosing spondylitis is associated with the anthrax toxin receptor 2 gene (ANTXR2). Ann Rheum Dis 2014; 73: 2054-2058.

[7] Yang X, Li M, Wang L, Hu Z, Zhang Y and Yang Q. Association of KIF21B genetic polymorphisms with ankylosing spondylitis in a Chinese Han population of Shandong Province. Clin Rheumatol 2015; 34: 1729-36.

[8] Davidson SI, Wu X, Liu Y, Wei M, Danoy PA, Thomas G, Cai Q, Sun L, Duncan E, Wang N, Yu Q, Xu A, Fu Y, Brown MA and Xu H. Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population. Arthritis Rheum 2009; 60: 3263-3268.

[9] Dong X, Zheng Y, Shi TY and Liu HY. Effects of tumor necrosis factor-alpha on sexual activity of male patients with ankylosing spondylitis. Clin Rheumatol 2015; 34: 915-920.

[10] Lv C, Wang Y, Wang J, Zhang H, Xu H and Zhang D. Association of Interleukin-10 gene polymorphisms with ankylosing spondylitis. Clin Invest Med 2011; 34: E370.

[11] Aguillon JC, Cruzat A, Cuenca J and Cuchacovich M. [Tumor necrosis factor alpha genetic polymorphism as a risk factor in disease]. Rev Med Chil 2002; 130: 1043-1050.

[12] Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C and Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. Hepatology 1999; 30: 526-530.

[13] Peng Y and Li LJ. TNF-alpha-308G/A polymorphism associated with TNF-alpha protein expression in patients with diabetic nephropathy. Int J Clin Exp Pathol 2015; 8: 3127-3131.

[14] Miteva LD, Stanilov NS, Deliysky TS and Stanilova SA. Significance of -1082A/G polymorphism of IL10 gene for progression of colorectal cancer and IL-10 expression. Tumour Biol 2014; 35: 12655-12664.

[15] van der Heijde D, Han C, DeVlam K, Burmester G, van den Bosch F, Williamson P, Bala M, Han J and Braun J. Infliximab improves productivity and reduces workday loss in patients with ankylosing spondylitis: results from a randomized, placebo-controlled trial. Arthritis Rheum 2006; 55: 569-574.

[16] Zauner D, Quehenberger F, Hermann J, Dejaco C, Stradner MH, Stojakovic T, Angerer H, Rinner B and Graninger WB. Whole body hyperthermia treatment increases interleukin 10 and toll-like receptor 4 expression in patients with ankylosing spondylitis: a pilot study. Int J Hyperthermia 2014; 30: 393-401.

[17] Israni AK, Li N, Cizman BB, Snyder J, Abrams J, Joffe M, Rebbeck T and Feldman HI. Association of donor inflammation- and apoptosis-related genotypes and delayed allograft function after kidney transplantation. Am J Kidney Dis 2008; 52: 331-339.

[18] Zhao M, Yang Y, Bi X, Yu X, Jia H, Fang H and Zang W. Acetylcholine Attenuated TNF-alpha-Induced Apoptosis in H9c2 Cells: Role of Calpain and the p38-MAPK Pathway. Cell Physiol Biochem 2015; 36: 1877-1889.

[19] Braun J, Deodhar A, Dijkmans B, Geusens P, Sieper J, Williamson P, Xu W, Visvanathan S, Baker D, Goldstein N and van der Heijde D. Efficacy and safety of infliximab in patients with ankylosing spondylitis over a two-year period. Arthritis Rheum 2008; 59: 1270-1278.

[20] Francois RJ, Neure L, Sieper J and Braun J. Immunohistological examination of open sacroiliac biopsies of patients with ankylosing spondylitis: detection of tumour necrosis factor alpha in two patients with early disease and transforming growth factor beta in three more advanced cases. Ann Rheum Dis 2006; 65: 713-720.

[21] Hohler T, Schaper T, Schneider PM, Meyer zum Buschenfelde KH and Marker-Hermann E. Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. Arthritis Rheum 1998; 41: 1489-1492.

[22] Manolova I, Ivanova M, Stoilov R, Rashkov R and Stanilova S. Association of single nucleotide polymorphism at position -308 of the tumor necrosis factor-alpha gene with ankylosing spondylitis and rheumatoid arthritis. Biotechnol Biotechnol Equip 2014; 28: 1108-1114.

[23] Ji Y, Yang X, Yang L, Wu D, Hua F, Lu T, Jia J, Ma C and Liang Q. Studies on correlation between

single-nucleotide polymorphisms of tumor necrosis factor gene and different stages of ankylosing spondylitis. Cell Biochem Biophys 2013; 67: 915-922.

[24] Kawamoto M, Matsui E, Kaneko H, Fukao T, Teramoto T, Kasahara K and Kondo N. IL-10 plays an important role as an immune-modulator in the pathogenesis of atopic diseases. Mol Med Rep 2008; 1: 837-842.

[25] Kim KW, Lee KE, Hong JY, Kim MN, Heo WI, Sohn MH and Kim KE. Involvement of IL-10 gene promoter polymorphisms in the susceptibility for childhood asthma. Lung 2011; 189: 417-423.

[26] Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI and Vandenbroucke JP. Genetic influence on cytokine production and fatal meningococcal disease. Lancet 1997; 349: 170-173.

[27] Zhang Z, Dai D, Yu K, Yuan F, Jin J, Ding L, Hao Y, Liang F, Liu N, Zhao X, Long J, Xi Y and Sun YY. Association of HLA-B27 and ERAP1 with ankylosing spondylitis susceptibility in Beijing Han Chinese. Tissue Antigens 2014; 83: 324-329.

[28] Nicknam MH, Mahmoudi M, Amirzargar AA, Jamshidi AR, Rezaei N and Nikbin B. HLA-B27 subtypes and tumor necrosis factor alpha promoter region polymorphism in Iranian patients with ankylosing spondylitis. Eur Cytokine Netw 2009; 20: 17-20.