Int J Clin Exp Med 2014;7(12):5877-5881
www.ijcem.com /ISSN:1940-5901/IJCEM0003863

Original Article

Genetic variants of STAT4 are associated with ankylosing spondylitis susceptibility and severity in a Chinese Han population

Zhixiang Liu, Peisen Zhang, Jie Dong

Department of Nuclear Medicine, The Hospital Affiliated of Weifang Medical University, Kuiwen Region 261031, Weifang City, Shandong Province, China

Received November 14, 2014; Accepted November 17, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Objective: Genetic factors play an important role in ankylosing spondylitis (AS) etiology and signal transducer and activator of transcription 4 (STAT4) gene polymorphisms may be involved. The aim of this study was to test whether STAT4 variants were associated with susceptibility to AS in a Chinese population. Methods: A total of 175 subjects who were diagnosed as AS and 249 healthy age-matched controls were enrolled in the present study. The rs7574865 G/T SNP in STAT4 gene was genotyped in all the subjects. The SPSS software was used to investigate the association between the rs7574865 genotypes and AS susceptibility or severity. Results: Rs7574865 G/T was found to be significantly associated with increased risk and severity of AS. Conclusion: Our data demonstrated the STAT4 rs7574865 G/T SNP was significantly associated with increased AS susceptibility and severity in Chinese Han Population.

Keywords: Ankylosing spondylitis, STAT4, gene polymorphism, Chinese Han population

Introduction

Ankylosing spondylitis (AS) is a subset of spondyloarthritis, which is characterized by chronic inflammatory arthritis that affects the spine and sacroiliac joints, causing characteristic inflammatory back pain, stiffness, and can lead to structural and functional impairments and a decrease in quality of life [1]. It is associated with a number of other features including peripheral arthritis, anterior uveitis, psoriasis and inflammatory bowel disease (IBD). The AS affects 0.55% of European and 0.23% of Chinese populations, but is uncommon in Africans and Japanese, and occurs more frequently in men than in women, at a ratio of 2:1 [2-4]. Whereas effective treatments are available that suppress inflammation and improve symptoms, there are not yet any treatments that have been shown to robustly slow the rate of ankylosis or induce disease remission.

Significant progress has been made in the genetics of AS in the past years, leading to new treatments in trial, and major leaps in understanding of the aetiopathogenesis of the disease. Ankylosing spondylitis is highly familial (sibling recurrence risk ratio of > 52%) and heritable (h^2 > 90%) [5, 6], with strong association with the human leucocyte antigen HLA-B27 gene. More than 80% of cases are positive for the HLA-B27 allele, but only a minority of HLA-B27 carriers develop ankylosing spondylitis (1-5%) [7, 8]. The low proportion of HLA-B27 carriers who develop ankylosing spondylitis reflects the fact that numerous other non-HLA-B27 variants are likely to influence disease susceptibility [5].

Signaling transducers and activators of transcriptions (STATs) are latent cytoplasmic transcription factors that induce the transcription of their target genes by recognizing specific DNA consensus sequences. The STAT4 gene that maps to chromosome 2q33 is expressed in activated peripheral blood monocytes, macrophages and dendritic cells at the sites of inflammation [9]. STAT4 transmits signals induced by interleukin-12 (IL-12), interleukin-23 (IL-23) and interferon-c (IFN-c), which are key cytokines in

Table 1. The summary of the basic characteristics of the groups

Clinical Characteristics	AS Patients	Controls	*P*-Value
No.	175	249	
Age (years)	28.3±9.2	27.1±6.8	n.s
Female/Male	54/121	77/172	n.s
Radiographic severity of AS			
1	42	0	
2	41	0	
3	54	0	
4	38	0	

the development of autoimmune diseases [10, 11]. Also, the STAT4 plays pivotal roles in the differentiation and proliferation of both T helper 1 (Th1) and T helper 17 (Th17) cells [11], which are crucial effectors in chronic inflammatory disorders. Given above, the STAT4 gene may play an important role in the pathogenesis of various autoimmune diseases like AS and rheumatoid arthritis (RA). To date, the SNP rs-7574865 in STAT4 gene has been reported to be associated with an increased risk for diverse complex autoimmune diseases in different ethnic populations, such as RA [12-16]. However, few studies were performed to investigate the relationship between STAT4 SNPs and AS susceptibility.

Accordingly, the aim of this study is to explore the association of STAT4 gene polymorphism (rs7574865) and the susceptibility and severity of AS. We performed genotyping analyses for STAT4 rs7574865 G/T with a case-control study in a Chinese Han population.

Method

The study was approved by the ethics committee of the affiliated hospital, and informed consent was obtained from patients and control participants.

Study population

A total of 175 patients diagnosed with AS and 249 age/sex-matched healthy controls who had no symptoms or signs of AS, other types of spondyloarthritis, and any autoimmune diseases were recruited in this study. All subjects included in this study were Chinese Han Population. The diagnosis of AS was based on the criteria of the American College of Rheumatology (modified New York criteria) [17]. Sacroiliitis was confirmed by computed tomography (CT). Radiographic severity of AS was graded by CT on a scale of 1-4 (grade 1: suspicious; grade 2: sclerosis, some erosions; grade 3: severe erosions, widening of the joint space, some ankylosis; and grade 4: complete ankylosis) [18]. The clinical and radiological assessment were performed by two independent examiners who were blinded to the clinical information. Disagreements were resolved through discussion and consensus. The control subjects were consecutively selected among individuals without a personal and family history of AS, other types of spondyloarthritis, or any autoimmune diseases.

Genotyping

DNA samples were obtained from all the participants from peripheral blood with the Chelex-100 method [19]. The SNPs were then genotyped using Taqman assay (Applied Biosystems 7500, ABI, Foster City, CA) and dual-labeled probes in real-time PCR. The primers and probes were designed and synthesized by Sigma (Sigma-Proligo, The Woodlands, TX). Genotyping was performed by independent laboratory personnel who were blinded to the study, and three authors independently reviewed the genotyping results, data entry, and statistical analyses. In addition, we randomly selected 5% samples of case and control subjects for reproducibility tests at least twice in different days and yielded a 100% concordant.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 16.0 for Windows was used in this study. The demographic and clinical data were presented as Mean ± SD and compared between groups by the Student's t-tests. The genotype and allelic frequencies were evaluated by Hardy-Weinberg equilibrium and compared by the Chi-square test. The association between the STAT4 rs7574865 polymorphism and AS susceptibility was assessed under the following genetic models, which were treated as a dichotomous variable: (i) T-allele versus G-allele for allele level comparison; (ii) GT + TT versus GG for a dominant model of the T allele; (iii) TT versus GT + GG for a recessive model of the T-allele; and (iv) TT versus GG for the extreme genotype. And the relationship between variants and the

Table 2. The genotype and allele distributions of the SNP rs7574865 for the cases and controls

Group	Allele (%)								H-WE
	GG	GT	TT	GT + TT	GT + GG	TT	G	T	
Control	130	102	17	119	232	17	72.7	27.3	0.616
Case	72	81	22	103	153	22	64.3	35.7	0.916
OR (95%CI)	/	/	1.34 (1.17 to 4.68)	1.56 (1.06 to 2.31)	/	1.96 (1.01 to 3.82)	/	1.48 (1.22 to 1.79)	/
P	/	/	0.0194	0.0297	/	0.0594	/	< 0.0001	/

Table 3. The association of SNP rs7574865 and radiographic severity (logistic regression analysis)

SNP	Allele	CT 1 MAF	CT 2 MAF	CT 3 MAF	CT 4 MAF	P	OR (95% CI)
rs7574865	T	26.6	33.3	36.4	48.3	0.002	1.92 (1.41-2.61)

radiographic severity of the disease was examined by logistic regression analysis. The $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

Demographic data of the population studied and the number of individuals in each group were shown in **Table 1**. There were no significant differences between groups in terms of age and gender. 23 cases were ascertained as grade 1 (suspicious), 51 as grade 2 (sclerosis, some erosions), 77 as grade 3 (severe erosions, widening of the joint space, some ankylosis), and 24 as grade 4 (complete ankylosis).

Association of STAT4 polymorphism (rs7574865) with AS

As expected, the distribution of the genotype of SNP rs7574865 conformed to the Hardy-Weinberg equilibrium and the genotyping success rate was 100%. **Table 2** listed the genotyped and allele distributions of the SNP rs7574865 for the cases and controls. The genotype frequencies of the STAT4 rs7574865 G/T polymorphism were 41.1% (GG), 46.3% (GT) and 12.6% (TT) in AS patients, and 52.2% (GG), 41.0% (GT) and 6.8% (TT) in controls (P=0.0302). For allele level comparison, the STAT4 rs7574865 T allele was associated with an increased risk of AS in terms of the frequency of allele comparison (T vs. G: OR=1.48; 95% CI=1.22-1.79, $P < 0.0001$). For a dominant model of the T allele, the GT + TT genotypes were associated with the risk for AS (GT + TT vs. GG, OR=1.56, 95% CI=1.06-2.31, P=0.0297). For a recessive model of the T allele, the TT homozygote genotype was not associated with susceptibility to AS (TT vs. GT + GG. OR=1.96, 95% CI=1.01-3.82, P=0.0594). For the extreme genotype, the TT genotypes were associated with the risk for AS (TT vs. GG, OR=1.34, 95% CI=1.17-4.68, P=0.0194).

We also analyzed the correlation between the STAT4 SNPrs 7574865 genotype and CT scores by logistic regression analysis, and found that rs7574865 was possibly linked to the radiographic severity of the disease (**Table 3**).

Discussion

AS involves interactions among genetic, environmental, and demographic factors, among which the genetic background is important determinants of AS. The most important finding of this study was that the STAT4 rs7574865 variant was significantly associated with increased AS susceptibility and radiographic severity in Chinese Han Population.

Much evidence from both animal models and humans has been accumulating to support the involvement of STAT4 in autoimmune diseases, including systemic lupus erythematosus, type I diabetes, primary Sjogren's syndrome, juvenile idiopathic arthritis, Crohn's disease, psoriasis, and RA. STAT4 is an important signaling molecule for IL-12, IL-23 and IFN-γ, which are key cytokines in the development of autoimmune diseases. Impaired autoimmune development was found in STAT4-deficient mice. It has also been found that STAT4 plays a crucial role in the function of innate and adaptive immune cells. For example, STAT4 was found to have a role in CD4+cell fate, to be necessary for generation of TH1 responses, and to play a role in TH17 cell differentiation, which made crucial effectors in chronic inflammatory disorders [10, 11, 20]. All these suggest the STAT4 may be an effective therapeutic target for autoimmune diseases.

The rs7574865 single nucleotide polymorphism (SNP) is located in the third intron of the STAT4 gene, it is considered to be responsible for splice variation or regulatory effects of STAT4, but its actual functional consequence remains to be identified [15]. A previous study reported rs7574865 SNP was in complete linkage disequilibrium (LD) with the other two strongest systemic lupus erythematosus (SLE) associated SNPs, indicating the relationship between rs7574865 SNP and autoimmune diseases like SLE [21]. Similar results were found that lupus patients with the minor rs7574865 allele of STAT4 exhibited lower levels of serum interferon-a, but stronger biological responses to this cytokine [22]. Also it was reported that the level of STAT4 expressed in peripheral blood mononuclear cell was correlated with the risk allele of rs7574865 [23]. Also, the SNP rs7574865 in STAT4 gene has been reported to be associated with an increased risk for RA in various studies. However, few studies were performed to investigate the relationship between STAT4 SNPs and AS susceptibility.

The most important limitation of the present study is the relatively small sample size. A single center case-control study is not sufficient to fully interpret the relationship between STAT4 polymorphisms and susceptibility to AS. Further study with multiple population and larger sample size is needed.

Conclusion

In conclusion, our data demonstrated the STAT4 rs7574865 SNP was found to be significantly associated with increased ankylosing spondylitis susceptibility and severity in Chinese Han Population.

Disclosure of conflict of interest

None.

Address correspondence to: Zhixiang Liu or Peisen Zhang, Department of Nuclear Medicine, The Hospital Affiliated of Weifang Medical University, 465 Yuhe Road, Kuiwen Region, Weifang City, Shandong province, China. E-mail: Liuzhixiang1105@126.com

References

[1] Braun J and Sieper J. Ankylosing spondylitis. Lancet 2007; 369: 1379-1390.

[2] Australo-Anglo-American Spondyloarthritis C, Reveille JD, Sims AM, Danoy P, Evans DM, Leo P, Pointon JJ, Jin R, Zhou X, Bradbury LA, Appleton LH, Davis JC, Diekman L, Doan T, Dowling A, Duan R, Duncan EL, Farrar C, Hadler J, Harvey D, Karaderi T, Mogg R, Pomeroy E, Pryce K, Taylor J, Savage L, Deloukas P, Kumanduri V, Peltonen L, Ring SM, Whittaker P, Glazov E, Thomas GP, Maksymowych WP, Inman RD, Ward MM, Stone MA, Weisman MH, Wordsworth BP and Brown MA. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 2010; 42: 123-127.

[3] Ng SC, Liao Z, Yu DT, Chan ES, Zhao L and Gu J. Epidemiology of spondyloarthritis in the People's Republic of China: review of the literature and commentary. Semin Arthritis Rheum 2007; 37: 39-47.

[4] Feldtkeller E, Khan MA, van der Heijde D, van der Linden S and Braun J. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. Rheumatol Int 2003; 23: 61-66.

[5] Brown MA, Laval SH, Brophy S and Calin A. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. Ann Rheum Dis 2000; 59: 883-886.

[6] 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.

[7] Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC and Sturrock RD. Ankylosing spondylitis and HL-A 27. Lancet 1973; 1: 904-907.

[8] Khan MA and Ball EJ. Genetic aspects of ankylosing spondylitis. Best Pract Res Clin Rheumatol 2002; 16: 675-690.

[9] Frucht DM, Aringer M, Galon J, Danning C, Brown M, Fan S, Centola M, Wu CY, Yamada N, El Gabalawy H and O'Shea JJ. Stat4 is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages at sites of Th1-mediated inflammation. J Immunol 2000; 164: 4659-4664.

[10] Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, Kapur R, Levy DE, Kansas GS and Kaplan MH. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol 2007; 178: 4901-4907.

[11] Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L and O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev 2004; 202: 139-156.

[12] Zhao Y, Liu X, Liu X, Su Y, Li Y, Zhang X, Zhu L, Wang S, Wang T, Jiang Q, Liu X, Li X, Huang C, Jia R, Lu X, Guo J and Li Z. Association of STAT4 gene polymorphism with increased susceptibility of rheumatoid arthritis in a northern

Chinese Han subpopulation. Int J Rheum Dis 2013; 16: 178-184.
[13] Tong G, Zhang X, Tong W and Liu Y. Association between polymorphism in STAT4 gene and risk of rheumatoid arthritis: a meta-analysis. Hum Immunol 2013; 74: 586-592.
[14] Mohamed RH, Pasha HF and El-Shahawy EE. Influence of TRAF1/C5 and STAT4 genes polymorphisms on susceptibility and severity of rheumatoid arthritis in Egyptian population. Cell Immunol 2012; 273: 67-72.
[15] Zervou MI, Sidiropoulos P, Petraki E, Vazgiourakis V, Krasoudaki E, Raptopoulou A, Kritikos H, Choustoulaki E, Boumpas DT and Goulielmos GN. Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. Hum Immunol 2008; 69: 567-571.
[16] Orozco G, Alizadeh BZ, Delgado-Vega AM, Gonzalez-Gay MA, Balsa A, Pascual-Salcedo D, Fernandez-Gutierrez B, Gonzalez-Escribano MF, Petersson IF, van Riel PL, Barrera P, Coenen MJ, Radstake TR, van Leeuwen MA, Wijmenga C, Koeleman BP, Alarcon-Riquelme M and Martin J. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. Arthritis Rheum 2008; 58: 1974-1980.
[17] van der Linden S, Valkenburg HA and Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984; 27: 361-368.
[18] Braun J, Golder W, Bollow M, Sieper J and van der Heijde D. Imaging and scoring in ankylosing spondylitis. Clin Exp Rheumatol 2002; 20: S178-184.
[19] Walsh PS, Metzger DA and Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 1991; 10: 506-513.
[20] Miossec P. Interleukin-17 in fashion, at last: ten years after its description, its cellular source has been identified. Arthritis Rheum 2007; 56: 2111-2115.
[21] Sigurdsson S, Nordmark G, Garnier S, Grundberg E, Kwan T, Nilsson O, Eloranta ML, Gunnarsson I, Svenungsson E, Sturfelt G, Bengtsson AA, Jonsen A, Truedsson L, Rantapaa-Dahlqvist S, Eriksson C, Alm G, Goring HH, Pastinen T, Syvanen AC and Ronnblom L. A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5. Hum Mol Genet 2008; 17: 2868-2876.
[22] Kariuki SN, Kirou KA, MacDermott EJ, Barillas-Arias L, Crow MK and Niewold TB. Cutting edge: autoimmune disease risk variant of STAT4 confers increased sensitivity to IFN-alpha in lupus patients in vivo. J Immunol 2009; 182: 34-38.
[23] Abelson AK, Delgado-Vega AM, Kozyrev SV, Sanchez E, Velazquez-Cruz R, Eriksson N, Wojcik J, Linga Reddy MV, Lima G, D'Alfonso S, Mig liaresi S, Baca V, Orozco L, Witte T, Ortego-Centeno N, group A, Abderrahim H, Pons-Estel BA, Gutierrez C, Suarez A, Gonzalez-Escribano MF, Martin J and Alarcon-Riquelme ME. STAT4 associates with systemic lupus erythematosus through two independent effects that correlate with gene expression and act additively with IRF5 to increase risk. Ann Rheum Dis 2009; 68: 1746-1753.