Int J Clin Exp Pathol 2015;8(1):933-937
www.ijcep.com /ISSN:1936-2625/IJCEP0003492

Original Article

Association of *PTPN22* polymorphsims and ankylosing spondylitis susceptibility

Qingxi Meng[1], Xiaojun Zhang[1], Xin Liu[1], Weiguo Wang[1], Peng Yu[2], Qunqun Shan[1], Zhaohu Mao[1], Tingbao Zhao[1]

[1]*Department of Spinal Cord Injury, Institute of Orthopedics and Traumatology of Chinese PLA, General Hospital of Jinan Military Area Command, Jinan, Shandong, China;* [2]*The First Veteran Institute of Jinan Military Region, Jinan, Shandong, China*

Received October 31, 2014; Accepted December 22, 2014; Epub January 1, 2015; Published January 15, 2015

Abstract: Background: As a susceptibility gene for AS, the polymorphsims of *PTPN22* associated with disease susceptibility. Methods: We selected two SNPs of rs1217406 and rs1217414 within *PTPN22* with Haploview software and investigated the relationship between the SNPs of *PTPN22* gene and AS susceptibility. 120 AS patients and 100 healthy people were enrolled from Qilu Hospital of Shandong University. And we genotyped the SNPs of *PTPN22* with PCR-RFLP method. Results: The results showed that C allele (rs1217406) and T allele (rs1217414) both were risk factors for AS (OR: 3.12, 2.13). The persons with A-T, C-C or C-T haplotypes were more likely to suffer AS (OR: 3.17, 3.66, 4.011). Conclusions: Due to the close relationship of *PTPN22* and AS, the study may be helpful for the early diagnosis and differential diagnosis.

Keywords: *PTPN22*, polymorphism, AS, susceptibility

Introduction

Ankylosing spondylitis (AS) is an autoimmune inflammatory disease with the clinical features of back pain and stiffness. The etiology of AS is still unknown. However, it is generally believed that genetic factor play an important role in the pathogenesis of AS. And the HLA region genes were reported to significantly associated with AS, of which human leukocyte antigen-B27 (HLA-B27) was the representative gene [1-4]. Moreover, previous studies showed that the expression level of interleukin 10 (IL10) was higher in the AS patients [5]. In addition, genes play crucial roles in regulating the expression of cytokines and immunomodulatory factors [6, 7]. Hence, we concluded that gene polymorphisms may influence the cytokine secretion pattern in patients with AS.

Protein tyrosine phosphatase nonreceptor 22 (*PTPN22)* gene encodes lymphoid protein tyrosine phosphatase (LYP) and plays a negatively regulatory role in T cell signal path. Some scholars believed that the improved activity of *PTPN22* could suppress TCR signal transduction of regulatory T cells, thus weakening the regulation function of T cells, so *PTPN22* dysfunction can lead to autoimmune diseases. Besides HLA genes, *PTPN22* is the most important susceptibility genes of human autoimmune disease and is an important candidate gene for AS in terms of autoimmune.

At present, several single nucleotide polymorphisms (SNPs) locus have been reported in *PTPN22* gene without inconsistent results. In our study, we performed gene detection of all participants including 120 AS patients and 100 healthy persons to explore whether there was relationship between polymorphisms (rs1217406, rs1217414) in promoter of *PTPN22* and AS susceptibility in Han Chinese of Shandong district.

Materials and methods

Subjects

The enrolled AS patients were from Qilu Hospital of Shandong University between 2008 to 2013 and all patients met the diagnostic criteria

Table 1. The characteristic of the cases and controls

Characteristic	Case (%)	Control (%)	*P* value
Age	27.49 ± 0.84	26.70 ± 0.83	0.504
Sex			0.555
Female	41 (34.17)	38 (38.00)	
Male	79 (65.83)	62 (62.00)	

revised in 1984 by America Rheumatism Association [8]. The participants were all Han Chinese with no blood relationship. The cases and controls were all signed the informed consent paper. All the subjects were checked and the person with other autoimmune diseases, endocrine diseases and tumor, chronic hepatitis and tuberculosis were excluded from the study.

Methods

Five ml venous blood were collected from the limosis patients and DNA was extracted with the method of EDTA anticoagulant, proteinase K digestion and salting out. The samples were stored under -20°C. 20μl PCR amplification reaction were prepared using 2× Taq PCR Masterix 10 μL, DNA template 1 μL, primer 1 μL (10 μmol/L) and DNase-free water. The PCR amplification was performed under the following conditions: 3 min 94°C initial denaturation followed by 30 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, then 72°C for 5 min. PCR products were identified with 150 g/L agarose gel electrophoresis. The primers of rs1217406 were: 5'-AAAGCCTTCACATTTTGGCATTATC-3' (forward), 5'-AGGCTTTTTCAGCGTCTTCCAACTC-3' (reverse). The primers of rs1217414 were: 5'-TATTGAGCACTTAGTATGTA-3' (forward), 5'-GCTTAGAACTGAACTTGGTA-3' (reverse).

Enzyme digestion reaction was 20 μL with PCR products 9 μL, RsaI (10 U/μL) 0.5 μL and were put in 37°C water bath overnight. Enzyme digestion products was detected by 250 g/L agarose gel.

Statistics

Hardy-Weinberg equilibrium (HWE) was used to evaluate the representativeness of the controls. And it was completed with χ^2 test. T-test was used to evaluate the average age differences between cases and controls. The difference of sex distribution was calculated by χ^2 test. Gene counting method was used in calculating gene and allele frequencies. Odds ratio (OR) and 95% confidence intervals (95% CI) were adopted to estimate the function of SNPs on AS susceptibility with logistic regression method. The tests were all two-tailed, and *P*-values < 0.05 were considered statistically significant. All the test was performed in SPSS 18.0.

Results

Subject characteristics

The average age of the cases was 27.49, while the control was 26.70. There was no significant differences in age between cases and controls (P = 0.504). In the study, male participants were more than female. Male accounted for 65.83% in the cases and 62.0% in the controls. However, there was no remark differences in sex distribution among the patients and healthy people (P = 0.555) (**Table 1**).

Correlation analysis of PTPN22 gene polymorphisms and AS susceptibility

With genotype frequencies of rs1217406 and rs1217414 (**Table 2**), we found that the genotype distribution in the control was in accordance with HWE. Hence, we concluded that the selected population was representative. And we also found that there were remark differences in genotype frequencies of rs1217406 and rs1217414 between cases and controls. OR and 95% CI were calculated to estimate the association between the genotypes and AS. The results suggested that CA (OR = 3.09, 95% CI = 1.67-5.74), CC (OR = 6.64, 95% CI = 2.92-15.10) genotypes of rs1217406 and TT (OR = 5.79, 95% CI = 1.26-26.66) genotype of rs1217414 all could increase the risk for AS. In addition, the C (rs1217406) and T (rs1217414) allele carriers were more likely to suffer AS (OR = 3.12, 95% CI = 2.08-4.69 OR = 2.13, 95% CI = 1.23-2.70).

Association analysis of haplotypes and AS susceptibility

Since the linkage disequilibrium between rs1217406 and rs1217414 (D' = 0.592, r^2 = 0.28), we investigated the functions of four haplotypes on the AS (**Table 3**). The A-C haplotype accounted for a large proportion in the cases (37.08%) and controls (68.00%). The fre-

Table 2. Association of genotypes and alleles and AS susceptibility

Genotype/allele	Case (%)	Control (%)	χ^2	P	OR (95% CI)
rs1217406					
AA	32 (26.67)	59 (59.00)	-	-	1
CA	52 (43.33)	31 (31.00)	13.133	0.000	3.09 (1.67-5.74)
CC	36 (30.00)	10 (10.00)	22.701	0.000	6.64 (2.92-15.10)
A	116 (48.33)	149 (74.50)	-	-	1
C	124 (51.67)	51 (25.50)	31.182	0.000	3.12 (2.08-4.69)
rs1217414					
CC	84 (70.00)	81 (81.00)	-	-	
CT	24 (20.00)	17 (17.00)	0.766	0.381	1.36 (0.68-2.72)
TT	12 (10.00)	2 (2.00)	6.286	0.012	5.79 (1.26-26.66)
C	192 (80.00)	179 (89.50)	-	-	1
T	48 (20.00)	21 (10.50)	7.446	0.006	2.13 (1.23-2.70)

Table 3. Association of haplotypes and AS susceptibility

Haplotype site 1-site 2	Case 2n = 240 (%)	Control 2n = 200 (%)	χ^2	*P* value	OR (95% CI)
A-C	89 (37.08)	136 (68.00)	-	-	1
A-T	27 (11.25)	13 (5.42)	10.775	0.002	3.17 (1.56-6.48)
C-C	103 (42.92)	43 (21.50)	34.062	0.000	3.66 (2.35-5.71)
C-T	21 (8.75)	8 (4.00)	11.297	0.001	4.01 (1.70-9.45)

quencies of C-T haplotype was 8.75% in the case and only 4.00% in the control. Further studies showed that A-T, C-C and C-T haplotypes all were significantly associated with AS susceptibility (OR = 3.17, 95% CI = 1.56-6.48; OR = 3.66, 95% CI = 2.35-5.71; OR = 4.01, 95% CI = 1.70-9.45).

Discussion

AS is a complex and polygenic disease based on genetic factors with more than 90% heritability [9]. Since the research on association of polymorphism of TNF-α and AS by Braun in 1995, researchers were increasingly concerned about the relationship of AS and genes [10]. Some scholars have begun to study the relationship of SNPs within *PTPN22* and AS [11-14].

Single nucleotide polumorphisms (SNP) is one special form of polymorphisms, occurred in genome sequences by a single base deletion or insertion, but more often is single base substitution. SNP is usually a mutant with the from of mutiple alleles or two alleles [15]. The close relationship of polymorphisms of *PTPN22* and inflammation diseases were confirmed and it suggested that SNPs of *PTPN22* may be causative factors for inflammation diseases including AS [16-28]. Due to the different regions and populations in the study, gene distributions were not exactly the same, thus resulting in the frequency deviation on the statistics. Our study specially selected Han population of Shandong area in China to study the correlation of *PTPN22* SNPs and AS susceptibility.

With the analysis of rs1217406 and rs1217414 in 100 healthy controls, we found genotype distributions of each loci were in accordance with HWE ($P = 0.06$; $P = 0.34$). And the genotype frequencies of rs1217406 and rs1217414 were significantly different between cases and controls. The CA and CC genotypes of rs1217406 both were risk factors for AS (OR: 3.09, OR = 6.64).In addition, the TT genotype of rs1217414 could increase the risk for AS (OR: 5.79). Further studies showed that the person with the C allele (rs1217406) or T allele (rs1217414) were more likely to suffer AS (OR: 3.12, 2.13). Four haplotypes of rs1217406 and rs1217414 were analyzed in the study and the results showed three haplotypes (A-T, C-C and C-T) were closely related to AS (OR: 3.17, 3.66, 4.011).

The above data could fully explain the relationship of the SNPs of *PTPN22* gene and AS, so we

concluded that there existed a close relationship between polymorphisms of *PTPN22* gene and AS susceptibility.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tingbao Zhao, Department of Spinal Cord Injury, Institute of Orthopedics and Traumatology of Chinese PLA, General Hospital of Jinan Military Area Command, 25 Shifan Road, Jinan 250031, Shandong, China. Tel: 86-15263498675; E-mail: zhanghuajng@126.com

References

[1] 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.

[2] Garcia-Medel N, Sanz-Bravo A, Alvarez-Navarro C, Gomez-Molina P, Barnea E, Marcilla M, Admon A and Lopez de Castro JA. Peptide handling by HLA-B27 subtypes influences their biological behavior, association with ankylosing spondylitis and susceptibility to ERAP1. Mol Cell Proteomics 2014; 13: 3367-80.

[3] Laval SH, Timms A, Edwards S, Bradbury L, Brophy S, Milicic A, Rubin L, Siminovitch KA, Weeks DE, Calin A, Wordsworth BP and Brown MA. Whole-genome screening in ankylosing spondylitis: evidence of non-MHC genetic-susceptibility loci. Am J Hum Genet 2001; 68: 918-926.

[4] Chou CT, Timms AE, Wei JC, Tsai WC, Wordsworth BP and Brown MA. Replication of association of IL1 gene complex members with ankylosing spondylitis in Taiwanese Chinese. Ann Rheum Dis 2006; 65: 1106-1109.

[5] Rudwaleit M, Siegert S, Yin Z, Eick J, Thiel A, Radbruch A, Sieper J and Braun J. Low T cell production of TNFalpha and IFNgamma in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. Ann Rheum Dis 2001; 60: 36-42.

[6] Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ and Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24: 1-8.

[7] Wilson AG, Symons JA, McDowell TL, McDevitt HO and Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997; 94: 3195-3199.

[8] 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.

[9] Mahmoudi M, Amirzargar AA, Jamshidi AR, Farhadi E, Noori S, Avraee M, Nazari B and Nicknam MH. Association of IL1R polymorphism with HLA-B27 positive in Iranian patients with ankylosing spondylitis. Eur Cytokine Netw 2011; 22: 175-180.

[10] Braun J, Bollow M, Neure L, Seipelt E, Seyrekbasan F, Herbst H, Eggens U, Distler A and Sieper J. Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. Arthritis Rheum 1995; 38: 499-505.

[11] Huang CH, Wei JC, Chen CC, Chuang CS, Chou CH, Lin YJ, Wang MF and Wong RH. Associations of the PTPN22 and CTLA-4 genetic polymorphisms with Taiwanese ankylosing spondylitis. Rheumatol Int 2014; 34: 683-691.

[12] Liu SS, Ding Y and Lou JQ. Potential role of PTPN22 in ankylosing spondylitis, comment on: associations of the PTPN22 and CTLA-4 genetic polymorphisms with Taiwanese ankylosing spondylitis. Rheumatol Int 2014; 34: 1417-1418.

[13] Tang L, Wang Y and Chen B. A variant within intron 1 of the PTPN22 gene decreases the genetic susceptibility of ankylosing spondylitis in a central south Chinese Han population. Scand J Rheumatol 2014; 43: 380-384.

[14] Zhang Q, Qi J, Hou S, Du L, Yu H, Cao Q, Zhou Y, Liao D, Kijlstra A and Yang P. A functional variant of PTPN22 confers risk for Vogt-Koyanagi-Harada syndrome but not for ankylosing spondylitis. PLoS One 2014; 9: e96943.

[15] 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.

[16] Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang SY, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ and Gregersen PK. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004; 75: 330-337.

[17] Rodriguez-Rodriguez L, Taib WR, Topless R, Steer S, Gonzalez-Escribano MF, Balsa A, Pascual-Salcedo D, Gonzalez-Gay MA, Raya E, Fernandez-Gutierrez B, Gonzalez-Alvaro I, Bottini N, Witte T, Viken MK, Coenen MJ, van Riel PL, Franke B, den Heijer M, Radstake TR,

Wordsworth P, Lie BA, Merriman TR and Martin J. The PTPN22 R263Q polymorphism is a risk factor for rheumatoid arthritis in Caucasian case-control samples. Arthritis Rheum 2011; 63: 365-372.

[18] Baca V, Velazquez-Cruz R, Salas-Martinez G, Espinosa-Rosales F, Saldana-Alvarez Y and Orozco L. Association analysis of the PTPN22 gene in childhood-onset systemic lupus erythematosus in Mexican population. Genes Immun 2006; 7: 693-695.

[19] Heward JM, Brand OJ, Barrett JC, Carr-Smith JD, Franklyn JA and Gough SC. Association of PTPN22 haplotypes with Graves' disease. J Clin Endocrinol Metab 2007; 92: 685-690.

[20] Serrano A, Marquez A, Mackie SL, Carmona FD, Solans R, Miranda-Filloy JA, Hernandez-Rodriguez J, Cid MC, Castaneda S, Morado IC, Narvaez J, Blanco R, Sopena B, Garcia-Villanueva MJ, Monfort J, Ortego-Centeno N, Unzurrunzaga A, Mari-Alfonso B, Sanchez-Martin J, de Miguel E, Magro C, Raya E, Braun N, Latus J, Molberg O, Lie BA, Moosig F, Witte T, Morgan AW, Gonzalez-Gay MA and Martin J. Identification of the PTPN22 functional variant R620W as susceptibility genetic factor for giant cell arteritis. Ann Rheum Dis 2013; 72: 1882-1886.

[21] Orozco G, Garcia-Porrua C, Lopez-Nevot MA, Raya E, Gonzalez-Gay MA and Martin J. Lack of association between ankylosing spondylitis and a functional polymorphism of PTPN22 proposed as a general susceptibility marker for autoimmunity. Ann Rheum Dis 2006; 65: 687-688.

[22] Palomino-Morales R, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Rodriguez L, Miranda-Filloy JA, Pascual-Salcedo D, Balsa A, Fernandez-Gutierrez B, Llorca J, Martin J and Gonzalez-Gay MA. Lack of association of PTPN22, STAT4 and TRAF1/C5 gene polymorphisms with cardiovascular risk in rheumatoid arthritis. Clin Exp Rheumatol 2010; 28: 695-701.

[23] Carlton VE, Hu X, Chokkalingam AP, Schrodi SJ, Brandon R, Alexander HC, Chang M, Catanese JJ, Leong DU, Ardlie KG, Kastner DL, Seldin MF, Criswell LA, Gregersen PK, Beasley E, Thomson G, Amos CI and Begovich AB. PTPN22 genetic variation: evidence for multiple variants associated with rheumatoid arthritis. Am J Hum Genet 2005; 77: 567-581.

[24] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ and Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559-575.

[25] Lee YH, Rho YH, Choi SJ, Ji JD, Song GG, Nath SK and Harley JB. The PTPN22 C1858T functional polymorphism and autoimmune diseases--a meta-analysis. Rheumatology (Oxford) 2007; 46: 49-56.

[26] Ban Y, Tozaki T, Taniyama M and Tomita M. The codon 620 single nucleotide polymorphism of the protein tyrosine phosphatase-22 gene does not contribute to autoimmune thyroid disease susceptibility in the Japanese. Thyroid 2005; 15: 1115-1118.

[27] Smith RL, Warren RB, Eyre S, Ke X, Young HS, Allen M, Strachan D, McArdle W, Gittins MP, Barker JN, Griffiths CE and Worthington J. Polymorphisms in the PTPN22 region are associated with psoriasis of early onset. Br J Dermatol 2008; 158: 962-968.

[28] Namjou B, Kim-Howard X, Sun C, Adler A, Chung SA, Kaufman KM, Kelly JA, Glenn SB, Guthridge JM, Scofield RH, Kimberly RP, Brown EE, Alarcon GS, Edberg JC, Kim JH, Choi J, Ramsey-Goldman R, Petri MA, Reveille JD, Vila LM, Boackle SA, Freedman BI, Tsao BP, Langefeld CD, Vyse TJ, Jacob CO, Pons-Estel B, Niewold TB, Moser Sivils KL, Merrill JT, Anaya JM, Gilkeson GS, Gaffney PM, Bae SC, Alarcon-Riquelme ME, Harley JB, Criswell LA, James JA and Nath SK. PTPN22 association in systemic lupus erythematosus (SLE) with respect to individual ancestry and clinical sub-phenotypes. PLoS One 2013; 8: e69404.