MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e919528
DOI: 10.12659/MSM.919528

Received: 2019.08.20
Accepted: 2019.11.27
Available online: 2020.03.05
Published: 2020.05.01

RUNX3 Polymorphisms Affect the Risk of Ankylosing Spondylitis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABC 1 Huawei Liu*
ABC 1 Ligong Fu*
BCG 1 Dawei He
CDE 1 Jiuzheng Deng
DF 1 Jianjin Zhu
AB 1 Kai Xu
DE 1 Dong Hu
BE 2 Jing Li
FG 2 Yan Wang
DE 2 Wenhao Hu
DE 1 Songhua Xiao

1 Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, P.R. China
2 Department of Orthopedics, Chinese People's Liberation Army (PLA) General Hospital, Beijing, P.R. China

* Huawei Liu, Ligong Fu contributed equally to this work

Corresponding Authors: Wenhao Hu, e-mail: jiotuifc@163.com, Songhua Xiao, e-mail: yybnrtg@yeah.net
Source of support: Departmental sources

Background: We aimed to assess the potential association of runt-related transcription factor 3 (*RUNX3*) gene variants with ankylosing spondylitis (AS) susceptibility among Chinese Han people.

Material/Methods: Genotyping for *RUNX3* variants was accomplished through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 115 AS patients and 102 healthy controls. Genotypes distributions of the polymorphisms in controls was assessed for their deviation from Hardy-Weinberg equilibrium (HWE). Moreover, odds ratio (OR) with 95% confidence interval (95%CI) was achieved using chi-square analysis to evaluate AS risk related to *RUNX3* polymorphisms. Additionally, logistic regression analysis produced adjusted OR values.

Results: Genotypes distribution of rs760805 and rs11249206 polymorphisms conformed to HWE in the control group ($P>0.05$). TT genotype of rs760805 appeared more frequently among AS cases than in controls ($P=0.033$), indicating its significant association with increased risk of AS onset (OR=2.309, 95%CI=1.069–4.892). The carriage of T allele in rs760805 also heightened AS incidence, in comparison to A allele (OR=1.578, 95%CI=1.075–2.316, $P=0.020$). Moreover, the carriage of AT+TT genotype in rs760805 and TT genotype in rs11249206 obviously increased risk of AS onset (OR=2.585, 95%CI=1.062–6.288).

Conclusions: *RUNX3* rs760805 polymorphism can contribute to AS incidence in Chinese Han people. The interaction of the 2 polymorphisms may be a risk factor for AS.

MeSH Keywords: Core Binding Factor Alpha 3 Subunit • Polymorphism, Single-Stranded Conformational • Protein Interaction Domains and Motifs • Spondylitis, Ankylosing

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/919528

1562 3 — 29

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Ankylosing spondylitis (AS) is the most common spondyloarthropathy (SpA). It is caused by chronic inflammatory disorders, presenting as spine fibrosis and poker spine and leading to damaged muscle, skeleton, and lung function [1,2]. It mainly affects young adults ages 18–22 years. AS imposes a heavy burden and huge economic loss on patients, families, and society [3]. Although drug treatment has been improved for AS, currently available medicines fail to achieve satisfactory effects [4]. The pathogenesis of AS remains unclear, but it is accepted that disease onset occurs by interactions between environmental and genetic factors [5,6]. Genome-wide association studies (GWASs) have identified numerous single-nucleotide polymorphisms (SNPs) related to AS susceptibility, such as those in *IL-23R*, *IL-17A*, and *BCL11B* genes [7,8].

Runt-related transcription factor 3 (*RUNX3*) belongs to the runt-domain transcription factor family and includes 3 members: Runx1, Runx2, and *Runx3* [9]. *RUNX3* can regulate the transcription of several genes involved in development, differentiation, immunity, and cancer [10,11]. *RUNX3* is involved in human autoimmune diseases and inflammation [12]. A case-control study by Apel et al. demonstrated that *RUNX3* was involved in psoriatic arthritis through a T cell-mediated mechanism [13]. Evans and colleagues also reported that variants in *RUNX3* were strongly associated with AS [14]. Vecellio et al. suggested that *RUNX3* is involved in AS via allele-specific effects on IRF4 enrollment, changing gene expressions [15]. However, there have been few studies on the association of *RUNX3* variants with AS incidence, especially in the Chinese population.

Therefore, in the present study, we assessed the genetic association of *RUNX3* variants with the risk of AS in Chinese Han people, and 2 common intronic polymorphisms (rs760805 and rs11249206) were selected.

Material and Methods

Subjects

In this case-control study, 115 AS patients (65 men and 50 women; mean age: 36.66±8.67 years) were diagnosed by a qualified rheumatologist based on x-ray, magnetic resonance imaging (MRI), or computed tomography (CT), and laboratory examinations at Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University. At the same time, 102 healthy individuals (52 men and 50 women; mean age: 35.51±9.47) were selected from the physical examination center of the hospital. The healthy individuals received medical examinations, and none of them had immune illnesses or other serious diseases. Healthy individuals with a family history of AS were excluded from our study. The controls were age- and sex-matched with the cases. All study subjects were unrelated Chinese Han people living in the Beijing region. Our research obtained permission from the Ethics Committees of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University. The participants knew the study objective, and signed the written informed consent before blood collection.

DNA extraction

We collected 2 ml of peripheral blood from each subject in the early morning into blood collection tubes with 0.5 mg/mL EDTA, and then them stored at –80°C until use. Whole-blood genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) following the manufacturer's instructions, and then stored at –20°C for later use.

Genotyping

Genotyping for SNPs rs760805 and rs11249206 in *RUNX3* was completed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primer sequences are listed in Table 1. The PCR system contained a volume of 25 μl mixture. PCR conditions were 34 cycles of 30 s at 95°C, 30 s at 63°C, and 1 min at 72°C, after a first denaturation of 5 min at 95°C, followed by a final extension at 72°C for 7 min.

Table 1. Primer sequences of *RUNX3* gene 2 polymorphisms rs760805 and rs11249206.

SNP		Primer sequences (5'-3')	Annealing temperature	Restriction enzyme
rs760805	For.	TCTCCCACTCAGTTCACAC	55.6°C	*Cla* I
	Rev.	CTGGCGCATTGAGCTGTA'		
rs11249206	For.	AAATGATTACTGGCCCATTTCTCATA	56.4°C	*Afa* I
	Rev.	TTCTCTTGGCCCCATTTCTG'		

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Genotype and allele distributions of *RUNX3* gene rs760805 and rs11249206 polymorphisms in case and control groups.

Genotype/allele	Case n=115(%)		Control n=102(%)		*P*	OR (95% CI)	*P**	OR (95% CI)*
rs760805								
AA	20	(17.39)	25	(24.51)	–	Ref	–	Ref
AT	50	(43.48)	52	(50.98)	0.609	1.202 (0.594–2.431)	0.615	1.199 (0.590–2.436)
TT	45	(39.13)	25	(24.51)	0.036	2.250 (1.047–4.834)	0.033	2.309 (1.069–4.892)
A	90	(39.13)	102	(43.27)	–	Ref	–	Ref
T	140	(60.87)	102	(56.73)	0.023	1.556 (1.062–2.278)	0.020	1.578 (1.075–2.316)
P_{HWE}			0.843					
rs11249206								
TT	45	(39.13)	41	(40.20)	–	Ref	–	Ref
CT	51	(44.35)	46	(45.10)	0.973	1.010 (0.565–1.806)	0.901	0.963 (0.532–1.745)
CC	19	(16.52)	15	(14.71)	0.725	1.154 (0.519–2.564)	0.783	1.121 (0.499–2.516)
T	141	(61.30)	128	(62.75)	–	Ref	–	Ref
C	89	(38.70)	76	(37.25)	0.758	1.063 (0.721–1.568)	0.847	1.040 (0.702–1.540)
P_{HWE}			0.721					

HWE – Hardy-Weinberg equilibrium; * OR and *P* values were adjusted by age and sex.

PCR products were examined by 1% agarose gel electrophoresis (AGE).

Subsequently, PCR products were digested by specific restriction enzymes (*Cla* I for rs760805 and *Afa* I for rs11249206). Then, the digested fragments for *RUNX3* rs760805 and rs11249206 polymorphisms were separated via 1.5% AGE and visualized under UV light.

Statistical analysis

Genotype and allele distributions for our selected polymorphisms were determined by direct counting. Genotype distribution in the controls was assessed for Hardy-Weinberg equilibrium (HWE) using chi-square analysis. Genotype and allele frequencies of these polymorphisms were compared between 2 groups through chi-square analysis. Odds ratio (OR) with corresponding 95% confidence interval (95%CI) was calculated to evaluate the relative risk of AS. Logistic regression analysis adjusted OR and 95%CI based on clinical features. SPSS 18.0 software (SPSS, Chicago, IL, USA) was used to perform statistical analyses. All *P* values were two-sided, and $P<0.05$ was regarded as statistically significant. Interaction between *RUNX3* polymorphisms was detected through cross analysis.

Results

HWE test

Genotype distribution for rs760805 and rs11249206 polymorphisms conformed to HWE in the control group ($P=0.843$ and 0.721, respectively), suggesting they were from the same mendelian population.

Distributions of *RUNX3* gene polymorphisms in individual groups

Allele and genotype frequencies of rs760805 and rs11249206 polymorphisms are shown in Table 2. For rs760805, the frequencies of AA, AT, and TT genotypes were 17.39%, 43.48%, 39.13%, respectively, in AS cases and 24.51%, 50.98%, 24.51%, respectively, in healthy controls. TT genotype of rs760805 appeared more frequently among the cases than in the controls ($P=0.036$), and after adjusting for age and sex, the difference remained significant ($P=0.033$), suggesting its association with increased risk of AS (OR=2.309, 95%CI=1.069–4.892). A and T allele frequencies of rs760805 were 39.13%, 60.87%, respectively, in cases, and 43.27% and 56.73%, respectively, in controls, indicating that the T allele increased the risk of AS onset (adjusted by clinical characteristics, $P=0.020$, OR=1.578, 95%CI=1.075–2.316), but no significant differences appeared between the 2 groups in genotypes or alleles frequencies of

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. The interaction of *RUNX3* rs760805 and rs11249206 polymorphisms in AS occurrence.

rs760805	rs11249206	Case (%)	Control (%)	*P*	OR (95%CI)
AA	TT	13 (11.30)	21 (20.59)		
AA	CT+CC	7 (6.09)	4 (3.92)	0.141	2.827 (0.690–11.577)
AT+TT	TT	32 (27.83)	20 (19.61)	0.034	2.585 (1.062–6.288)
AT+TT	CT+CC	63 (54.78)	57 (55.88)	0.142	1.785 (0.891–3.891)

rs11249206 polymorphism (*P*>0.05). After adjusting for age and sex, the results were similar to the unadjusted ones (*P*>0.05).

Interaction analysis of *RUNX3* polymorphisms in AS

The interaction between *RUNX3* rs760805 and rs11249206 polymorphisms was also analyzed for its role in AS susceptibility (Table 3). We found that, in comparison to the combination of AA genotype in rs760805 with TT genotype in rs11249206, the AT+TT genotype in rs760805 together with TT genotype in rs11249206 significantly increased the risk of AS onset (OR=2.585, 95%CI=1.062–6.288), but not for other combinations (*P*>0.05).

Discussion

AS is a highly heritable inflammatory arthritis, and mainly affects the axial skeleton, sacroiliac joints, and spine attachment points [16]. Despite its unclear etiology, AS has been linked to some risk factors, such as smoking, alcohol abuse, and inappropriate exercise habits [17]. In addition, genetic factors also play important roles in AS. HLA-B27 is a well-known genetic factor for AS occurrence, and more than 80–90% of AS patients are HLA-B27-positive [18]. Multiple genes and relevant polymorphisms are associated with AS etiology, but the exact pathogenesis of AS remains very unclear.

RUNX3 is located on chromosome 1p36.1 and functions as a tumor suppressor against various cancers [19]. *RUNX3* also takes part in immune and inflammatory responses. *RUNX3* can regulate the proliferation and activation of CD8 T cells in the thymus [20], and it can affect the differentiation of T helper type 1 (Th1) and Th2 cells, and regulate the Th1/Th2 balance [21]. Additionally, *RUNX3* may be correlated with B cell development [22]. For these reasons, we hypothesized that *RUNX3* is associated with AS. Vecellio et al. reported that *RUNX3* expression was lower in mononuclear cells in peripheral blood from AS cases compared with healthy controls [23]. Genetic variants in the *RUNX3* gene appear to influence individual susceptibility to AS [23–25]. Recent data show that up to 40% of binding sites for transcription factors are located in the introns region in genes [26]. Rs760805 and rs11249206 polymorphisms are located in intron 3 and 1 in the *RUNX3* gene, and they appear to influence the gene expression and function. Moreover, the influences of these 2 polymorphisms have been reported in several other diseases. For instance, Wu et al. revealed that rs760805 and rs11249206 polymorphisms can modulate the risk of gastric cancer in the Chinese population [27], and are also correlated with bladder cancer risk [28]. However, the genetic association of rs760805 and rs11249206 polymorphisms with AS has rarely been reported previously.

In our research, we investigated genetic influences of *RUNX3* rs760805 and rs11249206 polymorphisms on AS susceptibility among Chinese Han people. The results showed that individuals carrying rs760805 TT genotype had a 2.309 times higher risk of AS occurrence than AA genotype carriers, and the T allele of rs760805 also obviously enhanced the disease incidence. Rs760805 can alter *RUNX3* expression or combine with other risk factors to cause pathological conditions [29]. There is no known relationship between rs11249206 polymorphism and risk of AS onset. In addition, we found a strong interaction between these 2 polymorphisms in AS occurrence. AT+TT genotype in rs760805 with TT genotype in rs11249206 can significantly increase the risk of AS compared to AA genotype in rs760805 combined with TT genotype in rs11249206. Our research for the first time revealed the role of *RUNX3* rs760805 and rs11249206 polymorphisms in AS risk. The results obtained in our study might be helpful for early screening of people at high-risk for AS in the Chinese Han population.

Some shortcomings in this study should be noted. First, the sample size was relatively small, and only Chinese Han people were included. Second, environmental factors were not taken into consideration. Additionally, the exact mechanism by which *RUNX3* polymorphism affects AS development was not investigated. Therefore, well-designed studies with larger sample sizes and multiple ethnic groups are required to solve these issues.

Conclusions

RUNX3 rs760805 polymorphism, but not rs11249206, can influence individual susceptibility to AS in the Chinese Han population. Interaction between rs760805 and rs11249206 polymorphisms appear to be associated with AS etiology.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Mermerci Baskan B, Pekin Dogan Y, Sivas F et al: The relation between osteoporosis and vitamin D levels and disease activity in ankylosing spondylitis. Rheumatol Int, 2010; 30: 375–81
2. Ma XF, Wang XD, Liu RR, Luan QX: Efficacy research of salazosulfamide in ankylosing spondylitis and NAT1 gene polymorphism. Exp Ther Med, 2017; 14: 2999–3003
3. Jia B, Qi X: The genetic association between polymorphisms in lymphotoxin-alpha gene and ankylosing spondylitis susceptibility in Chinese group: A case-control study. Medicine (Baltimore), 2017; 96: e6796
4. Machado MA, Ferre F, Moura CS et al: Costs of drug therapy in patients with ankylosing spondylitis in Brazil. Rheumatol Ther, 2016; 3: 353–61
5. Huang F, Gu J, Liu Y et al: Efficacy and safety of celecoxib in chinese patients with ankylosing spondylitis: A 6-week randomized, double-blinded study with 6-week open-label extension treatment. Curr Ther Res Clin Exp, 2014; 76: 126–33
6. O'Rielly DD, Uddin M, Rahman P: Ankylosing spondylitis: Beyond genome-wide association studies. Curr Opin Rheumatol, 2016; 28: 337–45
7. Yang B, Xu Y, Liu X et al: IL-23R and IL-17A polymorphisms correlate with susceptibility of ankylosing spondylitis in a Southwest Chinese population. Oncotarget, 2017; 8: 70310–16
8. Karami J, Mahmoudi M, Amirzargar A et al: Promoter hypermethylation of BCL11B gene correlates with downregulation of gene transcription in ankylosing spondylitis patients. Genes Immun, 2017; 18: 170–75
9. Nadarajan N, Balasubramanian LK, Kuppannan S et al: Runt-related transcription factor 3: Single nucleotide polymorphism rs760805, gene expression, and methylation status in *Helicobacter pylori*-infected patients for determination of gastric cancer risk. J Gastrointest Cancer, 2013; 44: 444–49.
10. Nishimura R, Hata K, Takahata Y et al: Regulation of cartilage development and diseases by transcription factors. J Bone Metab, 2017; 24: 147–53
11. Lotem J, Levanon D, Negreanu V et al: Runx3 in immunity, inflammation and cancer. Adv Exp Med Biol, 2017; 962: 369–93
12. Fainaru O, Woolf E, Lotem J et al: Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. EMBO J, 2004; 23: 969–79
13. Apel M, Uebe S, Bowes J et al: Variants in RUNX3 contribute to susceptibility to psoriatic arthritis, exhibiting further common ground with ankylosing spondylitis. Arthritis Rheum, 2013; 65: 1224–31
14. Evans DM, Spencer CC, Pointon JJ et al: Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet, 2011; 43: 761–67
15. Vecellio M, Roberts AR, Cohen CJ et al: The genetic association of RUNX3 with ankylosing spondylitis can be explained by allele-specific effects on IRF4 recruitment that alter gene expression. Ann Rheum Dis, 2016; 75: 1534–40
16. Mahmoudi M, Jamshidi AR, Karami J et al: Analysis of killer cell immunoglobulin-like receptor genes and their HLA ligands in Iranian patients with ankylosing spondylitis. Iran J Allergy Asthma Immunol, 2016; 15: 27–38
17. Ahsan T, Erum U, Jabeen R, Khowaja D: Ankylosing spondylitis: A rheumatology clinic experience. Pak J Med Sci, 2016; 32: 365–68
18. van der Linden SM, Valkenburg HA et al: The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. A comparison of relatives of spondylitis patients with the general population. Arthritis Rheum, 1984; 27: 241–49
19. Chuang LS, Ito Y: RUNX3 is multifunctional in carcinogenesis of multiple solid tumors. Oncogene, 2010; 29: 2605–15
20. Woolf E, Xiao C, Fainaru O et al: Runx3 and Runx1 are required for CD8 T cell development during thymopoiesis. Proc Natl Acad Sci USA, 2003; 100: 7731–36
21. Kohu K, Ohmori H, Wong WF et al: The Runx3 transcription factor augments Th1 and down-modulates Th2 phenotypes by interacting with and attenuating GATA3. J Immunol, 2009; 183: 7817–24
22. Whiteman HJ, Farrell PJ: RUNX expression and function in human B cells. Crit Rev Eukaryot Gene Expr, 2006; 16: 31–44
23. Vecellio M, Cortes A, Roberts AR et al: Evidence for a second ankylosing spondylitis-associated RUNX3 regulatory polymorphism. RMD Open, 2018; 4: e000628
24. Cho SM, Jung SH, Chung YJ: A variant in RUNX3 is associated with the risk of ankylosing spondylitis in Koreans. Genomics Inform, 2017; 15: 65–68
25. Liu J, Lian Z, Xiao Y et al: Analysis of clinical indexes and RUNX3, TBKBP1, PPARGC1B polymorphisms in Chinese Han patients with ankylosing spondylitis. Genet Test Mol Biomarkers, 2015; 19: 37–43
26. Euskirchen G, Royce TE, Bertone P et al: CREB binds to multiple *loci* on human chromosome 22. Mol Cell Biol, 2004; 24: 3804–14
27. Wu D, Tian Y, Gong W et al: Genetic variants in the Runt-related transcription factor 3 gene contribute to gastric cancer risk in a Chinese population. Cancer Sci, 2009; 100: 1688–94
28. Zhang Z, Wang S, Wang M et al: Genetic variants in RUNX3 and risk of bladder cancer: A haplotype-based analysis. Carcinogenesis, 2008; 29: 1973–78
29. Hishida A, Matsuo K, Goto Y et al: Significant association of RUNX3 T/A polymorphism at intron 3 (rs760805) with the risk of gastric atrophy in *Helicobacter pylori* seropositive Japanese. J Gastroenterol, 2009; 44: 1165–71

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]