**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 ($P > 0.05$). 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 may 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
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]. Vecello 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 55.6°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. TCTCCACCTCAGTTTACAC | 55.6°C                | Cla I             |
|           | Rev. CTGGGCGATTAGCTGTA   |                       |                   |
| rs11249206| For. AAATGATTACTGGCCCATTTTCCTACA | 56.4°C               | Afa I             |
|           | Rev. TTCTCTGGCCCATTTTCCTCAG |                       |                   |
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 \textit{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 \textit{RUNX3} polymorphisms was detected through cross analysis.

Table 2. Genotype and allele distributions of \textit{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) |

\( P_{\text{HWE}} \) 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)       | 0.756| 1.063 (0.721–1.568) | 0.847| 1.040 (0.702–1.540) |

\( P_{\text{HWE}} \) 0.721

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

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 \textit{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
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.

### 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)  | 0.141| 2.827 (0.690–11.577) |
| AA            | CT+CC       | 7 (6.09) | 4 (3.92)    | 0.141| 1.785 (0.891–3.891) |
| 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) |
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