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# DNA methylation and transcriptome signature of the IL12B gene in ankylosing spondylitis

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ABSTRACT

*Objective:* Ankylosing spondylitis (AS) is an autoimmune disease without a reliable biomarker. This study investigated the IL12B gene methylation as a robust marker by integrating DNA methylation and mRNA data.
*Methods:* A two-stage design was used for methylome and transcriptome investigation. The first phase detected methylation level from 99 AS patients and 99 healthy controls (HCs) whilst the second phase measured mRNA level from 20 patients and 20 HCs. We conducted analysis of differential methylation sites and receiver operating characteristic (ROC) as well as mRNA level to verify methylation.
*Results:* We investigated 37 methylation sites that were mapped to 2 CpG islands (IL12B-1 and IL12B-2). Compared with HCs, the two islands were hypermethylated (IL12B-1: $P = 4.6 * 10\hat{} - 4$; IL12B-2: $P = 1.3 * 10\hat{} - 5$) and the mRNA level was overexpressed ($P = 0.004$) in AS patients. The subgroup analysis results showed a significant hypermethylation of the two islands in B27 positive group (IL12B-1: $P = 3.7 * 10\hat{} - 4$; IL12B-2: $P = 3.7 * 10\hat{} - 6$) and in male patients (IL12B-1: $P = 4.9 * 10\hat{} - 4$; IL12B-2: $P = 7.2 * 10\hat{} - 6$). ROC results found that the IL12B-1 island had a sensitivity of 62.6% and a specificity of 66.7%, and the IL12B-2 had a sensitivity of 50.0% and a specificity of 77.7%.
*Conclusion:* DNA methylation and transcriptome signature of the IL12B gene can discriminate AS patients from HCs, and hypermethylation of the IL12B may contribute to the pathogenesis of AS.

## 1. Introduction

Ankylosing spondylitis (AS) is a chronic complex autoimmune disease associated with inflammation and new bone formation in axial skeleton [1,2]. It is more prevalent in male individuals and has a male-to-female ratio of 2:1 [3–5]. Molecular Genetics has rapidly improved our understanding of occurrence and progression of AS, and many polymorphisms associated with the pathogenesis of AS have been identified [6–8]. For example, the susceptibility and severity of AS are strongly associated with the major histocompatibility complex (MHC) haplotype B27 [7,9,10]. However, the exact mechanism of AS is still unclear. Moreover, most patients with AS are sporadic rather than familial, indicating that additional mechanisms in the pathogenesis of AS are needed to be investigated. Epigenetic factors have recently emerged as underlying factors in the interpretation of complex diseases [11]. DNA methylation is an epigenetic modification that results from the addition of a methyl group to a cytosine DNA base in the middle of cytosine-guanine dinucleotide (CpG) and is most commonly observed in promoter. Increasing evidence suggested that DNA methylation plays an important role in the mechanism of autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and ankylosing spondylitis (AS) [11–17]. For example, genome-wide DNA methylation profile analysis detected 1915 differentially methylated CpG sites in AS, and the HLA-DQB1 gene was the most significant signal [18]. Moreover, candidate gene methylation studies identified some aberrant methylation changes. Comparing AS with healthy controls

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(HCs), hypermethylation of the BCL11B gene CpG3 and CpG5 was associated with increased risk of AS [15]. Methylation of the SOCS-1 gene was detected in the serum of B27-positive AS patients [19] and methylation of the DNMT1 promoter was significantly increased in AS patients [20]. Although these results are significant and interesting, reliable diagnostic biomarkers for AS are limited. More importantly, previous epigenetic association studies focused on the methylation level alone and lacked the integrating data of DNA methylation with mRNA level to thoroughly explore the functional role of DNA methylation in AS.

Recently, our teamwork and others identified that polymorphisms of the IL12B gene were associated with AS susceptibility and disease activity [21–24]. To identify novel AS associated methylation sites, we performed a two-stage case-control study (methylome and transcriptome) to evaluate the role of IL12B gene DNA methylation and mRNA expression in patients with AS. Our study suggests that the IL12B methylation may serve as a helpful marker and provides a useful clue for the diagnosis and treatment of AS.

## 2. Materials and methods

### 2.1. Human subjects

A two-stage case-control design was carried out in the Chinese Han population, and a total of 198 subjects (AS: HCs = 99:99) and 40 subjects (AS: HCs = 20:20) were enrolled for the testing of DNA methylation and mRNA expression respectively. All patients were collected from the First Affiliated Hospital of Anhui Medical University and met the 1984 modified New York criteria of the American College of Rheumatology, and HCs were recruited from the community hospitals and matched by age and gender with AS. All patients were asked to complete a questionnaire to provide their demographic characteristics and clinical characteristics. For example, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Score (ASDAS) were used for evaluating the disease activity, and Bath Ankylosing Spondylitis Functional Index (BASFI) was used for assessing the bodily functions. This study was approved by the ethics committee of Anhui Medical University and all participants signed informed consent.

### 2.2. DNA methylation detection

Genomic DNA was extracted from peripheral blood of AS and HCs using the QIAGEN kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, and the DNA samples were stored at minus 80 °C before DNA methylation test. DNA samples were quantified and then diluted to a concentration of 20 ng/μl. CpG islands adjacent to the IL12B promoter regions were chosen from 2 k upstream of transcriptional start site (TSS) to 1 k downstream of the first exon according to following criteria: (1) > 200 bp length; (2) cytosine-guanine content not < 50%; (3) ≥0.60 ratio of observed/expected dinucleotides CpG. Finally, 37 CpG methylation sites on the two islands (IL12B-1 and IL12B-2) were sequenced.

Bisulfite conversion of 400 ng genomic DNA was conducted using the EZ DNA Methylation™-GOLD Kit (ZYMO RESEARCH, CA, USA). Firstly, the samples with the rate of DNA bisulfite conversion < 98% were filtered out. PCR amplicons of target CpG regions were separated by agarose electrophoresis and purified using QIAquick Gel Extraction kit (QIAGEN, Hilden, Germany), and IL12B methylation assay was performed using Illumina Hiseq/Miseq 2000 according to the manufacturer's protocol. The primer sequences of IL12B methylation are as follows: IL12B-1 island, forward: 5′-GGTTGGAGGGAGGAAGTGT-3′, reverse: 5′-AATCCACTTCTCCATCCCTACTC-3′; IL12B-2 island, forward: 5′-GAGTAGGGATGGAGAAGTGGATT-3′; reverse: 5′-ATATAAAC CCCCCCAT CCC-3′. The methylation level of the IL12B gene was analyzed by MethylTarget™ (Genesky Biotechnologies Inc., Shanghai, China).

### 2.3. Quantitative Real-time PCR (qRT-PCR)

Ficoll-Hypaque density gradient centrifugation method was applied to isolate peripheral blood mononuclear cells (PBMCs) from peripheral blood of AS and HCs. Total cellular RNA was extracted using miRNeasy Mini Kit (Qiagen, Germany), quantification and concentration of RNA was determined using NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and then RNA was reverse-transcribed into complementary DNA (cDNA) using a PrimeScript™ RT reagent kit (Takara Bio Inc., Japan). The IL12B mRNA expression level was measured in the quantitative Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Green kit (Takara Bio Inc., Japan). The relative expression level of IL12B mRNA was normalized to the internal control β-actin and was calculated using $2^{-\Delta Ct} * 10\hat{}3$. The primer sequences of IL12B and β-actin are as follows: IL12B, forward: 5′-TAAGATGCGAGGCCAAGAATTA-3′, reverse: 5′-TACTCATACTCCTTGTTGTCCC-3′; β-actin, forward: 5′-TACT CATACTCCTTGTTGTCCC-3′, reverse: 5′-AGTTGAAGGTAGTTTCGTG GAT-3′.

### 2.4. Statistical analysis

The Kolmogorov-Smirnov test was used to check the normal distribution of the data. Normal distribution variables were presented as mean ± standard deviation (SD), and skewed variables were expressed as median with interquartile range (IQR). Student's $t$ and Mann-Whitney $U$ tests were used to compare the means of normal and asymmetric variables respectively. Pearson chi-square test was used to find the relationship among the categorical variables. Spearman's rank correlation coefficient test was used to explore the correlations between the IL12B gene and clinical characteristics. Binary logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs), and R software was used to plot the forest regression. Receiver operating characteristic (ROC) curve and area under curve (AUC) were used to evaluate the predictive power or feasibility of the IL12B gene as a biomarker for AS. All statistical analyses were performed by SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) and diagrams were generated using GraphPad Prism version 5.01 (GraphPad Software, Inc., CA, USA). A two-tailed value of $P < 0.05$ was considered significant. In the subgroup analysis of gender and HLA-B27 antigen, the Bonferroni method was applied for multiple comparisons and $P < 0.025$ was considered statistically significant. In addition, AS patients and HCs were stratified by the same factors in the gender subgroup analysis, while HCs were stratified by included all individuals in the B27 status stratified comparison.

## 3. Results

### 3.1. Demographic and clinical specifications

In the first stage, a total of 99 AS patients and 99 HCs were enrolled to measure DNA methylation. The mean ages of AS and HCs were 31.1 (SD: 9.9) and 31.8 (SD: 8.7) respectively. There were 83 (83.8%) male patients in AS group and 77 (77.8%) male individuals in HCs group, and there were no significant differences in gender and age between the two groups (Gender: $P = 0.353$; Age: $P = 0.635$). In the second stage of the transcriptome, a total of 20 AS patients and 20 HCs were included to measure the IL12B mRNA level. Compared with HCs group, the age and number of male patients in AS group were not statistically significant (AS age: 31.6 (7.9) and HCs age: 31.9 (7.6), $P = 0.919$; AS gender: 14 cases (70.0%) and HCs gender: 15 individuals (75%), $P = 0.723$). The details of ESR, CRP, BASFI, BASDAI, and ASDAS in AS patients are listed in the Table 1.

**Table 1**
Characteristics of subjects in two stage.

| | Methylation stage | qRT-PCR stage |
|---|---|---|
| | (AS = 99, HC = 99) | (AS = 20, HC = 20) |
| HC | | |
| Age (year) | 31.8 ± 8.7 | 31.9 ± 7.6 |
| Gender (male, %) | 83 (83.8) | 15 (75.0) |
| AS | | |
| Age (year) | 31.1 ± 9.9 | 31.6 ± 7.9 |
| Gender (male, %) | 77 (77.8) | 14 (70.0) |
| BMI (kg/m$^2$) | 22.58 ± 4.36 | 22.55 ± 3.03 |
| HLA-B27 (+, %) | 83 (83.8) | |
| ESR (mm/L) | 15.5 (27.5) | 10.0 (27.0) |
| CRP (m/L) | 8.9 (22.4) | 6.7 (30.0) |
| Disease duration (month) | 5.0 (7.0) | 64.0 (87.0) |
| BASFI (cm) | 0.9 (2.0) | 0.8 (2.5) |
| BASDAI (cm) | 2.1 (2.3) | 2.4 (1.5) |
| ASDAS (cm) | 3.0 (2.0) | 2.1 (1.3) |
| Global pain (cm) | 2.0 (3.8) | 0 (3.0) |
| Night pain (cm) | 2.0 (4.0) | 0 (4.0) |
| FFD (cm) | 6.0 (19.0) | 7.8 (14.8) |
| Chest expansion (cm) | 3.0 (3.0) | 3.5 (3.5) |
| Schober test (cm) | 6.0 (6.0) | 7.0 (5.0) |
| Occipito wall gap (cm) | 0 (2.3) | 0 (1.5) |

AS, Ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FFD, Finger-floor distance; HC, Health control.
Normal variables were presented as means ± standard deviation (SD), skewed data were expressed as median (interquartile range).

### 3.2. Methylation level of IL12B

The methylation level of CpG site was calculated by the proportion of methylated cytosine to the total tested cytosines. A total of 15 CpG sites in the IL12B-1 island and 22 CpG sites in the IL12B-2 island were detected to be methylated. Among the 37 methylation sites, CpG 3, CpG 8, CpG 10, CpG 13 and CpG 15 in the IL12B-1 island and CpG 18, CpG 23 and CpG34 in the IL12B-2 island were significantly methylated in AS patients compared with the HCs (Fig. 1A & B, all $P < 0.05$). In order to further understand the overall methylation status of the IL12B gene in AS patients, the CpG island methylation level was determined by calculating the methylation levels of all CpG sites. Compared with HCs group, hypermethylation of the IL12B gene was established in both IL12B-1 island and IL12B-2 island in AS patients ($P$ value of the IL12B-1 and IL12B-2 was $4.6 * 10\hat{}-4$ and $1.3 * 10\hat{}-5$, respectively; Fig. 1C & D). To evaluate the potentiality of the IL12B gene methylation as a biomarker for AS, ROC curve analysis was performed on the two CpG islands. The AUC of ROC curve was 0.6548 (95% CI: 0.5873–0.7313; $P = 0.0002$) for the IL12B-1 island, 0.6271 (95% CI: 0.5492–0.7050; $P = 0.0020$) for the IL12B-2 island. Further analysis of the diagnostic performance of CpG island revealed that the IL12B-1 could differentiate AS and HCs with a sensitivity of 62.6% and a specificity of 66.7%, and the IL12B-2 with a sensitivity of 50.0% and a specificity of 77.7% (Fig. 1E & F and Table 2).

### 3.3. Subgroup analysis of IL12B methylation

It is well known that the most important heritability of AS is from the B27 antigen and the male population [5,25,26]. Therefore, we performed subgroup analysis to further explore the role of IL12B methylation level in AS. In the B27 positive group, the methylation levels of IL12B-1 and IL12B-2 islands were significantly enhanced in AS patients than those in HCs ($P$ value of IL12B-1 and IL12B-2 was $3.7 * 10\hat{}-4$ and $3.7 * 10\hat{}-6$, respectively; Fig. 2A & B), but there were no significant differences between AS and HCs in the B27 negative group (Bonferroni adjustment of $P$ value $> 0.025$; Fig. 2C & D). In the subgroup analysis of gender, the hypermethylation of the IL12B gene was not detected in female patients (Bonferroni adjustment of $P$ value $> 0.05/2$; Fig. 2G & H) but in male patients ($P$ value of IL12B-1 and IL12B-2 was $4.9 * 10\hat{}-4$ and $7.2 * 10\hat{}-6$, respectively; Fig. 2E & F).

### 3.4. mRNA expression level of IL12B

To verify the methylation results, we measured the IL12B mRNA expression level in a cohort of 20 AS patients and 20 HCs using qRT-PCR assay. The relative expression level of IL12B gene was higher in AS patients than that in HCs (Fig. 3). ROC results showed that the AUC was 0.5675 (95% CI: 0.3864–0.7486; $P = 0.4652$) for the IL12B mRNA level (Table 2).

### 3.5. Methylation level and clinical characteristics

To investigate the association between the IL12B methylation and disease activity in AS, we analyzed the effect of methylation level on clinical characteristics (Table 3). The results showed that the IL12B-1 CpG island level was negatively associated with CRP ($r_s = -0.205$, $P = 0.043$) and was positively correlated with chest expansion ($r_s = -0.278$, $P = 0.009$). The methylation of IL12B-2 island was positively associated with Schober test ($r_s = -0.244$, $P = 0.027$). There was no significant difference between the IL12B mRNA and clinical characteristics.

## 4. Discussion

The IL12B gene is one of the most important genes that affect the IL-23 signal pathway in the pathogenesis of AS [23,27], and the polymorphisms of IL12B gene are associated with AS susceptibility [21–24,28]. Understanding the molecular mechanisms of epigenetics sheds a new light on the pathogenesis of AS and forms a novel therapeutic strategy. In this study, our findings indicated that DNA methylation levels of the IL12B gene in the peripheral blood may contribute to the diagnosis and evaluation of AS. Methylation level of the IL12B promoter was higher in AS patients than that in HCs and the difference was more significant in B27 positive individuals and male patients. The specificity and sensitivity of IL12B methylation in discriminating between AS and HCs were very meaningful. Moreover, DNA hypermethylation of IL12B was verified by transcriptome of mRNA expression.

Epigenetic modification serves as a dynamic association between genotype, environment and phenotype [11]. DNA methylation is the best characterized epigenetic modification and has become a new research hotspot in autoimmune disease. Epigenome-wide association study (EWAS) is a powerful technique for detecting methylation associated with the occurrence and development of disease. Previous EWAS identified 1915 different expression methylation CpG sites using Illumina Infinium HumanMethylation450 BeadChip [18]. However, EWAS has some unexpected challenges. For example, it does not cover all risk sites and lacks detailed clinical data to explore the relevance of methylation to clinical characteristics, so more accurate detection technologies have irreplaceable roles in methylation research. Some studies have shown that methylation of the DNMT1 and BCL11B gene was significantly higher in AS patients [15,20]. Furthermore, their studies indicated that differentially expressed genes influence AS progression through change methylation levels of target genes [15,20]. In the current study, in order to determine the methylation level of the IL12B gene, we measured the mRNA expression level of IL12B in the second transcriptome stage. We found that the levels of methylation and mRNA were significantly increased in AS patients than those in HCs, suggesting that genetics and epigenetics of the IL12B gene may be related to the pathogenesis of AS. Numerous studies have been investigated to

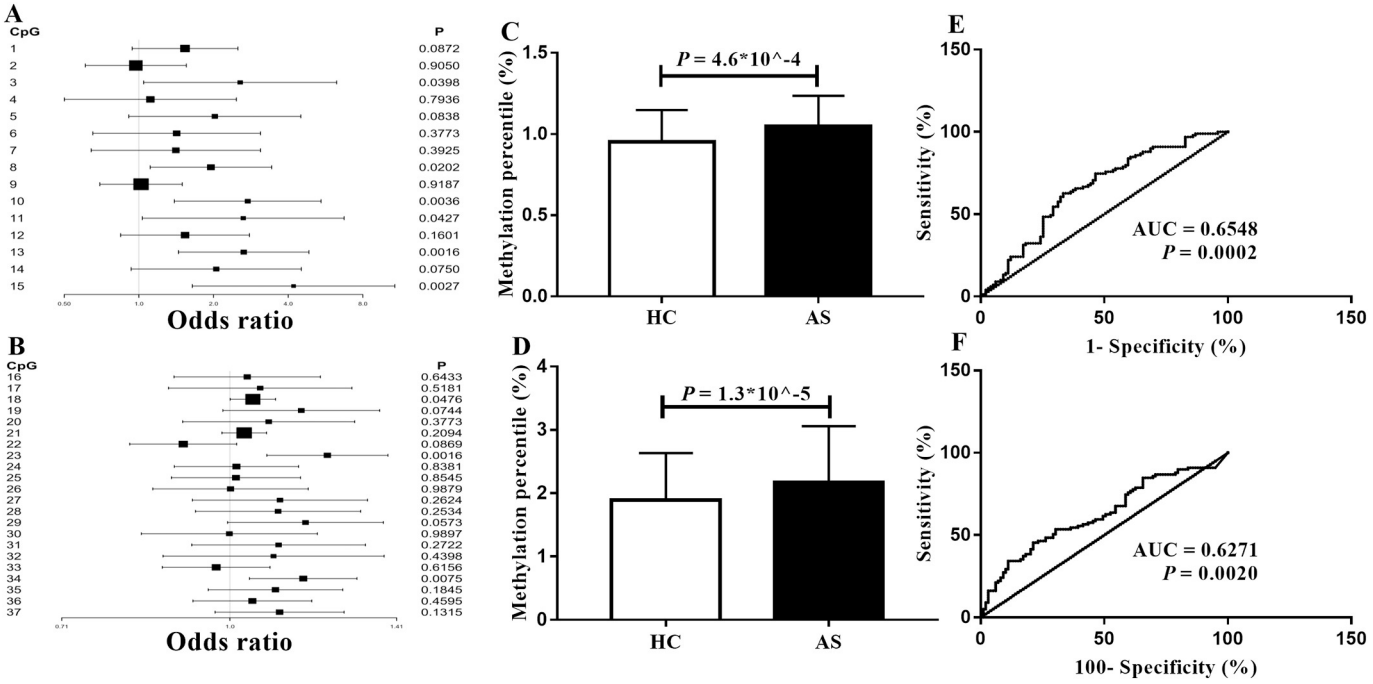


**Fig. 1.** Results of IL12B methylation and ROC analysis. (A) Methylation of 15 CpG sites in the IL12B-1 island, 6 of which are enhanced. The *squares* and *horizontal lines* correspond to the odds ratio (OR) and 95% confidence interval (CI) respectively. (B) Methylation of 22 CpG sites in the IL12B-2 island, 3 of which are enhanced. The *squares* and *horizontal lines* correspond to the OR and 95% CI respectively. (C) Methylation level of IL12B-1 island, which was determined by measuring the methylation levels of all CpG sites. (D) Methylation level of the IL12B-2 island. (E) ROC result of IL12B-1 island. (F) ROC result of IL12B-2. $P < 0.05$ was considered significant.

explore the association of IL12B polymorphisms with AS susceptibility. The rs6871626 variant was associated with AS susceptibility and disease activity in the Chinese population [21,23]. The IL12B gene can increase the production of IL-23 cytokines by encoding IL-12p40 and further stimulate the release of other proinflammation cytokines [27–29], thereby participating in the development of AS [22]. Therefore, we speculate that the susceptibility and progression of AS are not limited to genetic variations but also include epigenetic regulations (methylation modification). Furthermore, the IL12B mRNA expression was partly regulated by post-transcriptionally [30], so it is necessary to explore the mechanisms that may be involved in the IL12B protein levels. In this study, our findings demonstrated that methylation of the IL12B was weakly associated with disease activity, and there was no difference between the mRNA level and clinical characteristics. These results are similar to the previous studies. For example, methylation and expression levels of DNMT1 and BCL11B genes were not associated with clinical manifestations [15,20]. It is illustrated that DNA methylation may not affect patients' disease activity and functional status. Therefore, clinical manifestations of AS may be caused by other factors, such as environment, polymorphisms and drug consumption. Since AS is strongly associated with B27 antigen and gender [5,25,26], we performed the corresponding stratified analysis. Hypermethylation level of the IL12B was found in B27 positive group and in male patients, and the possible explanation is that the sample sizes in male patients and in B27 positive individuals are larger than that in female and in B27 negative population respectively. Moreover, heterogeneous methylation of the IL12B gene may contribute to the differential distribution of gender and B27 antigen in AS patients. Another study found that methylation of the SOCS-1 gene was detected in the B27-positive patients but not in the B27-positive HCs [19]. Intriguingly, compared with the previous methylation studies [11,16–20], we first explored the diagnostic value of DNA methylation levels in AS patients by ROC curve analysis and the result of AUC value was > 60%. These results indicated that the IL12B methylation can serve as a potential novel biomarker to distinguish AS patients from HCs.

This study considers several limitations. Firstly, our design is a single-center case-control study that requires a multicenter, larger sample size study to validate. Secondly, subjects in the methylome and transcriptome are from two separate groups, so the correlation analysis of DNA methylation and mRNA level is not performed. Finally, DNA methylation and RNA level are significantly higher in AS patients, but protein expression of the IL12B is not measured, thus further studies on the role of protein level in the pathogenesis of AS are needed.

Our findings demonstrated that the IL12B gene is significantly

**Table 2**
The diagnosis value of methylation and mRNA of IL12B gene.

| | AUC (95% CI) | Sensitivity (%) | Specificity (%) | Cutoff point (%) | *P* |
|---|---|---|---|---|---|
| IL12B-1 CpG | 0.6548 (0.5873–0.7313) | 62.6 | 66.7 | 0.978 | 0.0002 |
| IL12B-2 CpG | 0.6271 (0.5492–0.7050) | 50.0 | 77.7 | 2.369 | 0.0020 |
| IL12 mRNA | 0.5675 (0.3864–0.7486) | 45.0 | 75.0 | 0.871 | 0.4650 |

AUC, Area under curve; CI, Confidence interval.

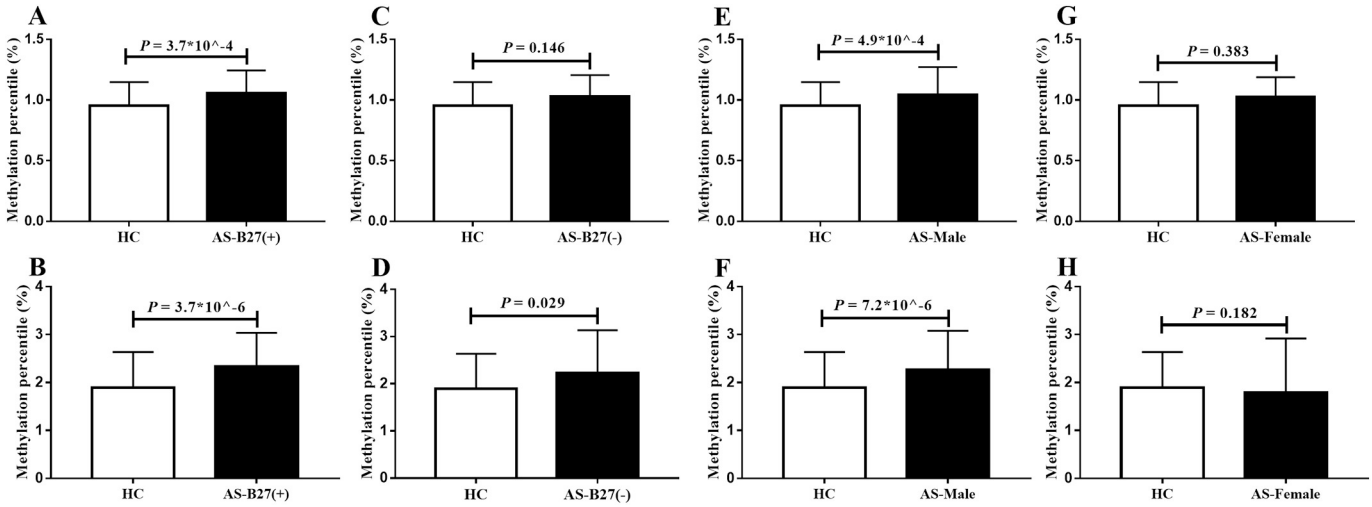


**Fig. 2.** Subgroup analysis of IL12B CpG island methylation based on HLA-B27 antigen and gender. (A) The IL12B-1 island methylation in the B27 positive group. (B) The IL12B-2 island methylation in the B27 positive group. (C) The IL12B-1 island methylation in the B27 negative group. (D) The IL12B-2 island methylation in the B27 negative group. (E) The IL12B-1 island methylation in male patients. (F) The IL12B-2 island methylation in male patients. (G) The IL12B-1 island methylation in female patients. (H) The IL12B-2 island methylation in female patients. $P < 0.025$ was considered significant.

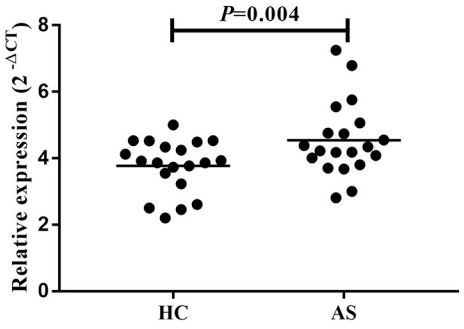


**Fig. 3.** The result of IL12B mRNA expression level. $P < 0.05$ was considered significant.

**Table 3**
Correlation between methylation and mRNA of IL12B and clinical characteristics.

| | IL12B-1 | | IL12B-2 | | IL12B mRNA | |
|---|---|---|---|---|---|---|
| | $r_s$ | P | $r_s$ | P | $r_s$ | P |
| ESR | −0.091 | 0.374 | 0.064 | 0.552 | 0.386 | 0.103 |
| CRP | −0.205 | 0.043 | −0.028 | 0.794 | 0.334 | 0.206 |
| BASFI | −0.021 | 0.839 | −0.045 | 0.673 | 0.217 | 0.358 |
| BASDAI | −0.013 | 0.897 | −0.174 | 0.104 | 0.053 | 0.825 |
| ASDAS | −0.121 | 0.237 | −0.174 | 0.103 | 0.223 | 0.346 |
| Global pain | 0.192 | 0.261 | 0.18 | 0.323 | 0.155 | 0.527 |
| Night pain | 0.125 | 0.461 | 0.325 | 0.065 | 0.146 | 0.55 |
| FFD | −0.19 | 0.073 | −0.068 | 0.547 | −0.234 | 0.32 |
| Chest expansion | 0.278 | 0.009 | −0.084 | 0.462 | −0.201 | 0.409 |
| Schober test | −0.012 | 0.913 | 0.244 | 0.027 | 0.079 | 0.748 |
| Occipito wall gap | −0.062 | 0.556 | 0.121 | 0.271 | −0.267 | 0.255 |

ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FFD, Finger-floor distance.
$r_s$, Spearman's rank correlation coefficient.

methylated in AS patients, especially in the B27 antigen-positive patients and in male individuals, and these results are verified at the mRNA level. We also found that IL12B methylation is essential in the diagnosis of AS patients, but the feasibility of IL12B methylation in predicting clinical characteristics warrants further investigation. Since methylation modification of the IL12B gene plays a critical role in the pathogenesis of AS, it can be considered as a useful diagnostic tool and may be a promising therapeutic biomarker in the future.

*Abbreviations*

| | |
|---|---|
| AS | Ankylosing spondylitis |
| AUC | Area under curve |
| ASDAS | Ankylosing Spondylitis Disease Activity Score |
| BASDAI | Bath Ankylosing Spondylitis Disease Activity Index |
| BASFI | Bath Ankylosing Spondylitis Functional Index |
| CRP | C-reactive protein |
| CpG | cytosine-guanine dinucleotide |
| cDNA | complementary DNA |
| CI | Confidence intervals |
| ESR | erythrocyte sedimentation rate |
| EWAS | Epigenome-wide association study |
| HC | Health control |
| IQR | Interquartile range |
| MHC | Major histocompatibility complex |
| OR | odds ratio |
| PBMC | Peripheral blood mononuclear cell |
| ROC | Receiver operating characteristic |
| RA | Rheumatoid arthritis |
| SLE | Systemic lupus erythematosus |
| SD | Standard deviation |
| TSS | Transcriptional start site |

## Ethics approval and consent to participate

This study was approved by the ethics committee of Anhui Medical University, with all the participants declaring their consent by signing.

## Consent for publication

All authors gave their consent for publication.

## Availability of data and material

The datasets are available from the corresponding author on reasonable request.

## Competing interests

All authors declare they have no conflicts of interest.

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## Authorship

Conception and design of the study: PF and ZX. Perform research: ZX, LJ, PZ and MY. Draft the article: PF and ZX. Analyze data: ZX, MY and LR. Acquisition of data: YS, YSY, DJ, SX and XS.

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## References

[1] M. Dougados, D. Baeten, Spondyloarthritis, Lancet 377 (2011) 2127–2137.
[2] Baeten D, Sieper J, Braun J, et al., Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis, NEJM. 373(2015):2534–48.
[3] Montoya J, Matta NB, Suchon P, et al., Patients with ankylosing spondylitis have been breast fed less often than healthy controls: a case-control retrospective study, Ann. Rheum. Dis. 75(2016):879–82.
[4] W. Xu, C.G. Liang, Y.F. Li, et al., Involvement of Notch1/Hes signaling pathway in ankylosing spondylitis, Int J Clin Exp Patho. 8 (2015) 2737–2745.
[5] Dean LE, Jones GT, MacDonald AG, et al., Global prevalence of ankylosing spondylitis. Rheumatology. 53(2014):650–7.
[6] Cai G, Zhang X, Xin L, et al., Associations between vitamin D receptor gene polymorphisms and ankylosing spondylitis in Chinese Han population: a case-control study. Osteoporosis Int. 27(2016):2327–2333.
[7] A. Cortes, S.L. Pulit, P.J. Leo, et al., Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1, Nat. Commun. 6 (2015) 7146.
[8] Cortes A, Gladman D, Raychaudhuri S, et al., Imputation-based analysis of MICA alleles in the susceptibility to ankylosing spondylitis. Ann. Rheum. Dis. 77(2018):1691–1692.
[9] J. Braun, J. Sieper, Ankylosing spondylitis, Lancet 369 (2007) 1379–1390.
[10] Cortes A, Hadler J, Pointon JP, et al., Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nat. Genet. 45(2013):730–8.
[11] H. Zhu, L.F. Wu, X.B. Mo, et al., Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells, Ann. Rheum. Dis. (2018), https://doi.org/10.1136/annrheumdis-2018-213970 Oct 8.
[12] Nakano K, Whitaker JW, Boyle DL, et al., DNA methylome signature in rheumatoid arthritis. Ann. Rheum. Dis. 72(2013):110–7.
[13] Zhao M, Zhou Y, Zhu B, et al., IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. Ann. Rheum. Dis. 75(2016):1998–2006.
[14] Ai R, Hammaker D, Boyle DL, et al., Joint-specific DNA methylation and transcriptome signatures in rheumatoid arthritis identify distinct pathogenic processes. Nat. Commun. 7(2016):11849.
[15] 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. 18(2017):170–175.
[16] U.M. Chandrasekharan, Z. Wang, Y. Wu, et al., Elevated levels of plasma symmetric dimethylarginine and increased arginase activity as potential indicators of cardiovascular comorbidity in rheumatoid arthritis, Arthritis Res Ther. 20 (2018) 123.
[17] W. Meng, Z. Zhu, X. Jiang, et al., DNA methylation mediates genotype and smoking interaction in the development of anti-citrullinated peptide antibody-positive rheumatoid arthritis, Arthritis Res Ther. 19 (2017) 71.
[18] J. Hao, Y. Liu, J. Xu, et al., Genome-wide DNA methylation profile analysis identifies differentially methylated loci associated with ankylosis spondylitis, Arthritis Res Ther. 19 (2017) 177.
[19] Lai NS, Chou JL, Chen GC, et al., Association between cytokines and methylation of SOCS-1 in serum of patients with ankylosing spondylitis. Mol. Biol. Rep. 41(2014):3773–80.
[20] Aslani S, Mahmoudi M, Garshasbi M, et al., Evaluation of DNMT1 gene expression profile and methylation of its promoter region in patients with ankylosing spondylitis. Clin. Rheumatol. 35(2016):2723–2731.
[21] L. Zhang, D. Fan, L. Liu, et al., Association study of IL-12B polymorphisms susceptibility with ankylosing spondylitis in mainland Han population, PLoS One 10 (2015) e0130982.
[22] Ivanova M, Manolova I, Miteva L, et al., Genetic variations in the IL-12B gene in association with IL-23 and IL-12p40 serum levels in ankylosing spondylitis. Rheumatol. Int. 2018. doi: https://doi.org/10.1007/s00296-018-4204-0
[23] W.F. Ruan, J.T. Xie, Q. Jin, et al., The diagnostic and prognostic role of interleukin 12B and interleukin 6R gene polymorphism in patients with ankylosing spondylitis, J. Clin. Rheumatol. 24 (2018) 18–24.
[24] Wong RH, Wei JC, Huang CH, et al., Association of IL-12B genetic polymorphism with the susceptibility and disease severity of ankylosing spondylitis. J. Rheumatol. 39(2012):135–40.
[25] Chen L, Ridley A, Hammitzsch A, et al., Silencing or inhibition of endoplasmic reticulum aminopeptidase 1 (ERAP1) suppresses free heavy chain expression and Th17 responses in ankylosing spondylitis. Ann. Rheum. Dis. 75(2016):916–23.
[26] Costantino F, Talpin A, Said-Nahal R, et al., A family-based genome-wide association study reveals an association of spondyloarthritis with MAPK14. Ann. Rheum. Dis. 76(2017):310–314.
[27] Coffre M, Roumier M, Rybczynska M, et al., Combinatorial control of Th17 and Th1 cell functions by genetic variations in genes associated with the interleukin-23 signaling pathway in spondyloarthritis. Arthritis Rheum. 65(2013):1510–21.
[28] Jadon D, Tillett W, Wallis D, et al., Exploring ankylosing spondylitis-associated ERAP1, IL23R and IL12B gene polymorphisms in subphenotypes of psoriatic arthritis. Rheumatology. 52(2013):261–6.
[29] D. Huang, M.R. Cancilla, G. Morahan, Complete primary structure, chromosomal localisation, and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit, Genes Immun. 1 (2000) 515–520.
[30] J.A. Warrington, U. Bengtsson, High-resolution physical mapping of human 5q31-q33 using three methods: radiation hybrid mapping, interphase fluorescence in situ hybridization, and pulsed-field gel electrophoresis, Genomics 24 (1994) 395–398.