The associations between PD-1, CTLA-4 gene polymorphisms and susceptibility to ankylosing spondylitis: a meta-analysis and systemic review

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Abstract Previous surveys had evaluated the effects of the PD-1, CTLA-4 gene polymorphisms on susceptibility to ankylosing spondylitis (AS), but the results remained controversial. To briefly examine these consequences, a comprehensive meta-analysis was conducted to estimate the relationships between PD-1 rs11568821, rs2227982, rs2227981, CTLA-4 +49 A/G and −318 C/T polymorphisms and AS risk. The available articles dated to December 2014 were searched in the PUBMED, MEDLINE and EMBASE databases. The data of the genotypes and/or alleles for the PD-1 rs11568821, rs2227982, rs2227981, CTLA-4 +49 A/G and −318 C/T polymorphisms in the AS and control subjects were extracted, and statistical analysis was conducted by STATA 11.2 software. Summary odds ratios (ORs) with their 95% confidence intervals (95% CIs) were calculated to determine the strength of associations with fixed-effects or random-effects models. A total of eight published studies were finally involved in this meta-analysis. Meta-analysis of PD-1 rs2227982 polymorphism under the T allele versus C allele (OR 1.744, 95% CI 1.477–2.059, P < 0.0001), TT+TC versus CC (OR 2.292, 95% CI 1.654–3.175, P < 0.0001), TT versus CC (OR 1.883, 95% CI 1.299–2.729, P = 0.001) revealed a significant association with AS. Our meta-analysis demonstrated that the rs2227982 polymorphism in the PD-1 gene might contribute to AS susceptibility. However, further studies with large sample sizes and among different ethnicity populations should be required to confirm this association.

Keywords PD-1 · CTLA-4 · Polymorphisms · Ankylosing spondylitis · Meta-analysis

Introduction

Ankylosing spondylitis (AS) is a chronic autoimmune disease that is characterized by pain and stiffness in the spine and sacroiliac joints, which causes the initial bone and joint, and eventually leads to AS [1]. The prevalence of AS in the Chinese population is 0.24% [2], whereas the median prevalence across studies in the Europeans is 18.6/10 000 [3]. Previous studies had confirmed that the genetic factors play an important role in the pathogenesis of AS [4]. The human leukocyte antigen (HLA)-B27 is the first genetic factor identified in this disease, and it confers the greatest susceptibility to AS (20–30 %) [5, 6]. However, HLA-B27 cannot explain all patients with AS, as only 5% of HLA-B27-positive individuals ultimately develop to AS [7], indicating that other non-HLA-B27 susceptibility genes could contribute to AS susceptibility.

The human programmed cell death 1 (PD-1) gene is located on chromosome 2q37.3 [8], which encodes a cell surface membrane protein. The PD-1 protein is a negative regulator of T cells [9] that belongs to the immunoglobulin (Ig) receptor superfamily. PD-1 is actively expressed in the cell surface during the activation of T and B cells. The cytoplasmic immunoreceptor tyrosine-based inhibitory
motif of PD-1 is activated by an interaction between PD-1 and its corresponding ligands, PDL-1 and PDL-2 (both B7 family ligands) [10, 11], which induces the inhibitory signal to inhibit the proliferation of T and B cells to maintain peripheral tolerance [10–12]. The vivo studies reported that blockage of PDL-1 and PDL-2 led to diabetes [13] and experimental autoimmune encephalomyelitis [14]. Therefore, blocking the interaction of PD-1 and its ligands may be associated with AS. Several polymorphisms in PD-1 gene had been identified in the previous study [9]. In AS, the most widely studied PD-1 polymorphisms were the rs11568821, rs2227982 and rs2227981. Several studies had found autoimmune genotypes mapping to the region of chromosome 2q33 that encodes cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). CTLA-4 is a critical regulatory molecule expressed in T cells, which plays a significant role in inhibiting T cell activation and in peripheral tolerance [15]. Diverse investigations had indicated that CTLA-4 might play an important role in the regulation of self-tolerance by the immune system and in regulating immune system involvement in the pathogenesis of many autoimmune diseases [16]. More than 100 polymorphisms had been identified in the CTLA-4 gene, and of these polymorphisms, CTLA-4 +49 A/G and −318 C/T polymorphisms had been best investigated in AS. However, the relationships between those polymorphisms and susceptibility to AS remain unclear; some studies on association between PD-1, CTLA-4 polymorphisms and AS risk had used comparatively small samples [17–24], and the results about the role of PD-1 or CTLA-4 gene polymorphisms in AS patients are inconsistent and inconclusive.

Due to majority studies only included a small sample size, each of them might have inadequate power to illuminate a positive association and lack the evidence to clarify an absence of connection. Furthermore, the low statistical powers of individual studies could explain the contradictory published results. On the other hand, meta-analysis is a powerful means to synthesize data from different investigations on the same issue. Therefore, we had performed a systematic review and meta-analysis of available documents. The aim of the present study was to identify whether these polymorphisms in the region of PD-1 or CTLA-4 were associated with the risk of AS.

Materials and methods

Study selection

The PUBMED, MEDLINE and EMBASE electronic databases were comprehensively searched, with the following terms utilized: “programmed cell death 1” or “PD-1” or “PDCD-1” and “cytotoxic T lymphocyte antigen 4” or “CTLA-4” and “ankylosing spondylitis” or “AS” and “polymorphism” or “genetic.” All documents were updated to December 2014. The language was limited to English. Additional relevant references quoted in searched documents were also selected.

Inclusion and exclusion criteria

We selected the studies meeting the following criteria: (1) case–control studies on the association between PD-1, CTLA-4 polymorphisms and AS risk; (2) comprised genotype data; (3) sufficient data for evaluating odds ratio (OR) with their 95 % confidence interval (95 % CI).

We excluded the studies if they satisfied the following criteria: (1) studies in which genotype frequencies or alleles could not be ascertained; (2) studies in which family members had been studied; (3) reviews or abstracts; (4) animal studies. (5) Unpublished studies should not be eligible for inclusion.

Data extraction

All data were independently and in duplicate extracted from all selected studies by two independent investigators (SC and CWD). Inter-researcher disagreements were resolved by consensus or by a third investigator (YZL). The following information was collected from each selected study: author, publication year, ethnicity of the subject population, total numbers of patients and controls, and the frequency of the genotypes and alleles of PD-1 and CTLA-4 polymorphisms in cases and controls.

Statistical analysis

The statistical significance of OR was assessed with Z test, and \( P < 0.05 \) was considered to be statistically significant. Using a fixed-effect model or random-effect model depended on the degree of heterogeneity among studies. The Cochran’s Q statistic and the \( I^2 \) statistic were applied to judge whether or not heterogeneity existed among the studies included in this meta-analysis. For \( Q \) test, \( P > 0.10 \) indicated lack of heterogeneity among studies, so that the combined OR evaluated of each investigation was calculated by the fixed-effect model. Otherwise, a random-effect model was used. The \( I^2 \) statistic was also calculated to evaluate heterogeneity, with \( I^2 < 25 \% \) considered as low heterogeneity, 25–50 % as moderate and >50 % as representative of statistically significant heterogeneity [25, 26]. Under the dominant model (AA+AG versus GG), recessive model (AA versus GG+AG), additive model (AA versus GG) and allele model (A versus G), we evaluated the strength of associations between PD-1, CTLA-4 polymorphisms and the risk of AS by calculating a pooled OR with their 95 %
CI. Sensitivity analysis was conducted by successively excluding one study to evaluate the stability of the outcomes [27]. The potential publication bias was evaluated using the Begg’s funnel plot [28]. Besides, Hardy–Weinberg equilibrium (HWE) in the controls was appraised by Chi-square test [29]. Statistical analysis was done using STATA 11.2 software (Stata Corporation, College Station, TX, USA). The powers of each study were computed as the probabilities of detecting associations between PD-1, CTLA-4 polymorphisms and AS at the 0.05 level of significance, assuming an OR of 1.5 (small effect size). The power analysis was performed using the statistical program G*Power (http://www.psycho.uni-duesseldorf.de/app/projects/gpower).

Results

Studies and populations characteristics

Through electronic and manual searching methods, we identified forty-one potentially relevant investigations and twenty of these were chose for full-text review based on title or abstract details [17–24, 30–41]. Twelve studies were excluded because either they were not about the association between PD-1, CTLA-4 polymorphisms and the risk of AS, or they contained no extractable data, other polymorphism data or review [30–41]. Finally, we enrolled a total of eight published articles, which met our inclusion criteria [17–24] (Fig. 1). A collective total of 919 AS patients and 982 controls were included across the five studies related to PD-1 gene polymorphisms and the susceptibility to AS. For CTLA-4 polymorphisms, there were only three articles including 683 cases and 615 controls. Since eight studies including in our meta-analysis were all conducted in Asians, we could not perform the ethnicity-specific meta-analysis. Relevant characteristics of the studies included in the meta-analysis are provided in Table 1. The statistical powers of these studies ranged from 56.5 to 97.7 % (Table 1).

Association of the PD-1 rs2227982, rs2227981 polymorphisms and susceptibility to AS

Table 2 shows the summary of the meta-analysis outcomes regarding the relationship between the PD-1 gene polymorphisms and AS risk. Meta-analysis was performed on the rs2227982 and rs2227981 polymorphisms. For rs2227982 polymorphism, there were five studies regarding this polymorphism and the risk of AS [17–21]. There was no evidence of heterogeneity under the allele model (T versus C: \( I^2 = 47.6 \% \), \( P_{\text{heterogeneity}} = 0.106 \)), the recessive model (TT versus CC: \( I^2 = 47.6 \% \), \( P_{\text{heterogeneity}} = 0.106 \)), the additive model (TT versus CC: \( I^2 = 47.6 \% \), \( P_{\text{heterogeneity}} = 0.106 \)) and the dominant model (TT versus TC: \( I^2 = 47.6 \% \), \( P_{\text{heterogeneity}} = 0.106 \)) (Table 1). Therefore, these three genetic models used fixed-effect model. However, the dominant model (TT+TC versus CC: \( I^2 = 52.6 \% \), \( P_{\text{heterogeneity}} = 0.077 \)) had apparently between-study heterogeneity. Thus, a random-effect model was used for the dominant model. Significantly increased AS risk was found for T allele versus C allele (OR 1.744, 95 % CI 1.477–2.059, \( P < 0.0001 \), Fig. 2a), for TT+TC versus CC (OR 2.292, 95 % CI 1.654–3.175, \( P < 0.0001 \), Fig. 2b), for TT versus CC (OR 1.883, 95 % CI 1.299–2.729, \( P = 0.001 \), Fig. 2c; Table 2). However, not significantly increased risk was found for TT versus CC+TC (OR 1.332, 95 % CI 0.937–1.895, \( P = 0.110 \), Fig. 2d; Table 2).

For rs2227981 polymorphism, three studies regarding this polymorphism and the susceptibility to AS were selected [17, 20, 21]. There was no evidence of heterogeneity under the allele model (T versus C: \( I^2 = 52.2 \% \), \( P_{\text{heterogeneity}} = 0.123 \)) and the dominant model (TT+TC versus CC: \( I^2 = 0.0 \% \), \( P_{\text{heterogeneity}} = 0.559 \)). Therefore, these two genetic models used fixed-effect model. However, the recessive model (TT versus CC+TC: \( I^2 = 75.7 \% \), \( P_{\text{heterogeneity}} = 0.016 \)) and the additive model (TT versus CC: \( I^2 = 70.3 \% \), \( P_{\text{heterogeneity}} = 0.034 \)) had apparently between-study heterogeneity. Thus, a random-effect model was used for the dominant model. Meta-analysis of the allele model, the dominant model, the recessive model and the additive model of rs2227981 polymorphism failed to reveal any association with AS (Table 2; Fig. 3).

Meta-analysis of the CTLA-4-308 T/C, +49 G/A polymorphisms and AS susceptibility

Table 2 shows the summary of the meta-analysis outcomes regarding the relationship between the CTLA-4 gene polymorphisms and susceptibility to AS. Meta-analysis was performed on the CTLA-4-308 T/C and +49 G/A polymorphisms. For CTLA-4-308 T/C polymorphism, there were only two studies regarding this polymorphism and the risk of AS [22, 23]. There was no evidence of heterogeneity under the allele model (T versus C: \( I^2 = 0.0 \% \), \( P_{\text{heterogeneity}} = 0.661 \)), the dominant model (TT+TC versus CC: \( I^2 = 0.0 \% \), \( P_{\text{heterogeneity}} = 0.595 \)), the recessive model (TT versus CC+TC: \( I^2 = 0.0 \% \), \( P_{\text{heterogeneity}} = 0.589 \)) and the additive model (TT versus CC: \( I^2 = 0.0 \% \), \( P_{\text{heterogeneity}} = 0.558 \)). Therefore, these four genetic models used fixed-effect model. Significantly increased AS risk was found for T allele versus C allele (OR 0.648, 95 % CI 0.430–0.976, \( P = 0.038 \), Fig. 4a; Table 2). However, analysis of the dominant model, the recessive model and the additive model of CTLA-4-308 T/C polymorphism failed to reveal any association with AS (Fig. 4b–d; Table 2).

For CTLA-4 +49 G/A polymorphism, three studies regarding this polymorphism and the susceptibility to AS were selected [22–24]. The allele model (G versus A: \( I^2 = 72.1 \% \), \( P_{\text{heterogeneity}} = 0.028 \)) and the dominant model
(GG+GA versus AA: $I^2 = 62.9\%$, $P_{\text{heterogeneity}} = 0.068$) had apparently between-study heterogeneity. Thus, a random-effect model was used for these two models. However, there was no evidence of heterogeneity under the recessive model (GG versus AA+GA: $I^2 = 44.3\%$, $P_{\text{heterogeneity}} = 0.166$) and the additive model (GG versus AA: $I^2 = 41.1\%$, $P_{\text{heterogeneity}} = 0.183$). Therefore, these two genetic models used fixed-effect model. Meta-analysis of the allele model, the dominant model, the recessive model and the additive model of CTLA-4+49 G/A polymorphism failed to reveal any association with AS (Table 2; Fig. 5).

Sensitivity analysis and publication bias

To further reinforce our conclusions, the sensitivity analysis was performed by consecutively excluding individual studies. For PD-1 rs2227982 polymorphism, the corresponding summary ORs were not changed significantly, indicating that our results were statistically robust (detailed data not shown) (Fig. 6). At the same time, the corresponding summary ORs were not changed significantly for PD-1 rs2227981, CTLA-4-308 T/C and +49 G/A polymorphisms (detailed data not shown).
### Table 1  Characteristics of the studies and populations included in the meta-analysis

<table>
<thead>
<tr>
<th>Author (references)</th>
<th>Countries (ethnicity)</th>
<th>Numbers</th>
<th>Case (genotype)</th>
<th>Control (genotype)</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>Association P value</th>
<th>PHWE</th>
<th>Power (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu [17]</td>
<td>China (A)</td>
<td>216</td>
<td>13</td>
<td>12</td>
<td>PD-1</td>
<td>rs2227982</td>
<td>0.004</td>
<td>0.272</td>
<td>87.3</td>
</tr>
<tr>
<td>Qian [18]</td>
<td>China (A)</td>
<td>255</td>
<td>38</td>
<td>12</td>
<td>PD-1</td>
<td>rs2227982</td>
<td>0.028</td>
<td>&lt;0.05</td>
<td>85.7</td>
</tr>
<tr>
<td>Soleimanifar [19]</td>
<td>Iran (A)</td>
<td>161</td>
<td>19</td>
<td>9</td>
<td>PD-1</td>
<td>rs2227982</td>
<td>0.08</td>
<td>0.804</td>
<td>76.8</td>
</tr>
<tr>
<td>Lee [20]</td>
<td>Korea (A)</td>
<td>95</td>
<td>3</td>
<td>3</td>
<td>PD-1</td>
<td>rs2227982</td>
<td>NA</td>
<td>0.0012</td>
<td>56.5</td>
</tr>
<tr>
<td>Yang [21]</td>
<td>China (A)</td>
<td>196</td>
<td>14</td>
<td>9</td>
<td>PD-1</td>
<td>rs2227981</td>
<td>0.025</td>
<td>0.960</td>
<td>87.3</td>
</tr>
<tr>
<td>Liu [17]</td>
<td>China (A)</td>
<td>216</td>
<td>26</td>
<td>22</td>
<td>PD-1</td>
<td>rs2227981</td>
<td>NA</td>
<td>0.040</td>
<td>56.5</td>
</tr>
<tr>
<td>Lee [20]</td>
<td>Korea (A)</td>
<td>95</td>
<td>7</td>
<td>26</td>
<td>PD-1</td>
<td>rs2227981</td>
<td>NA</td>
<td>0.711</td>
<td>78.3</td>
</tr>
<tr>
<td>Azizi [23]</td>
<td>Asian</td>
<td>196</td>
<td>14</td>
<td>14</td>
<td>PD-1</td>
<td>rs2227981</td>
<td>0.452</td>
<td>0.172</td>
<td>61.4</td>
</tr>
<tr>
<td>Lee [24]</td>
<td>Taiwan (A)</td>
<td>142</td>
<td>2</td>
<td>3</td>
<td>PD-1</td>
<td>rs11568821</td>
<td>NA</td>
<td>0.272</td>
<td>87.3</td>
</tr>
<tr>
<td>Soleimanifar [19]</td>
<td>Iran (A)</td>
<td>161</td>
<td>3</td>
<td>3</td>
<td>PD-1</td>
<td>rs11568821</td>
<td>0.45</td>
<td>0.804</td>
<td>76.8</td>
</tr>
<tr>
<td>Azizi [23]</td>
<td>Iran (A)</td>
<td>150</td>
<td>10</td>
<td>10</td>
<td>CTLA-4</td>
<td>+49</td>
<td>0.055</td>
<td>0.009</td>
<td>61.4</td>
</tr>
<tr>
<td>Lee [24]</td>
<td>Taiwan (A)</td>
<td>142</td>
<td>56</td>
<td>39</td>
<td>CTLA-4</td>
<td>+49</td>
<td>0.212</td>
<td>0.172</td>
<td>63.1</td>
</tr>
<tr>
<td>Huang [22]</td>
<td>Taiwan (A)</td>
<td>391</td>
<td>148</td>
<td>168</td>
<td>CTLA-4</td>
<td>+49</td>
<td>0.317</td>
<td>0.156</td>
<td>97.7</td>
</tr>
</tbody>
</table>


a Power calculations assume \( \alpha = 0.05 \), OR = 1.5
Table 2  Meta-analysis of associations between the PD-1 and CTLA-4 polymorphisms and AS

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Comparison</th>
<th>Number of studies</th>
<th>Test of association</th>
<th>Test of heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1 rs2227982</td>
<td>T versus C (allele)</td>
<td>5</td>
<td>1.744</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>TT+TC versus CC (dominant)</td>
<td>5</td>
<td>2.292</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>TT versus CC+TC (recessive)</td>
<td>5</td>
<td>1.332</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>TT versus CC (additive)</td>
<td>5</td>
<td>1.883</td>
<td>0.001</td>
</tr>
<tr>
<td>PD-1 rs2227981</td>
<td>T versus C (allele)</td>
<td>3</td>
<td>1.135</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td>TT+TC versus CC (dominant)</td>
<td>3</td>
<td>1.201</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>TT versus CC+TC (recessive)</td>
<td>3</td>
<td>0.981</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td>TT versus CC (additive)</td>
<td>3</td>
<td>1.091</td>
<td>0.832</td>
</tr>
<tr>
<td>CTLA-4 -308</td>
<td>T versus C (allele)</td>
<td>2</td>
<td>0.648</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>TT+TC versus CC (dominant)</td>
<td>2</td>
<td>0.662</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>TT versus CC+TC (recessive)</td>
<td>2</td>
<td>0.323</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>TT versus CC (additive)</td>
<td>2</td>
<td>0.309</td>
<td>0.129</td>
</tr>
<tr>
<td>CTLA-4 +49</td>
<td>G versus A (allele)</td>
<td>3</td>
<td>1.109</td>
<td>0.558</td>
</tr>
<tr>
<td></td>
<td>GG+GA versus AA (dominant)</td>
<td>3</td>
<td>1.132</td>
<td>0.643</td>
</tr>
<tr>
<td></td>
<td>GG versus AA+GA (recessive)</td>
<td>3</td>
<td>0.943</td>
<td>0.628</td>
</tr>
<tr>
<td></td>
<td>GG versus AA (additive)</td>
<td>3</td>
<td>0.950</td>
<td>0.783</td>
</tr>
</tbody>
</table>

Bold values indicate statistically significant P values

OR odds ratio, CI confidence interval, F fixed-effects model, R random-effects model

Fig. 2  Forest plot of the susceptibility to AS associated with PD-1 rs2227982 polymorphism. a T versus C, forest plot; b TT+TC versus CC, forest plot, c TT versus CC+TC, d TT versus CC
Fig. 3  Forest plot of the susceptibility to AS associated with PD-1 rs2227981 polymorphism. a T versus C, forest plot; b TT+TC versus CC, forest plot, c TT versus CC+TC, d TT versus CC.

Fig. 4  Forest plot of the susceptibility to AS associated with CTLA-4-308 T/C polymorphism. a T versus C, forest plot; b TT+TC versus CC, forest plot, c TT versus CC+TC, d TT versus CC.
To examine publication bias, Begg’s funnel plot and Egger’s test were performed for the association between PD-1 rs2227982, rs2227981, CTLA-4-308 T/C and +49 G/A polymorphisms and AS risk. The shape of the funnel plots was almost symmetrical. The Egger’s test and Begg’s test indicated that there was no evidence of publication bias (Egger’s test \( P = 0.126 \); Begg’s test \( P = 0.221 \) for PD-1 rs2227982 T versus C, Fig. 7).

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**Fig. 5** Forest plot of the susceptibility to AS associated with CTLA-4-49 G/A polymorphism. a G versus A, forest plot; b GG+GA versus AA, forest plot, c GG versus AA+GA, d GG versus AA

**Fig. 6** Results of sensitivity analysis from random-effects estimates. (PD-1 rs2227982 polymorphism: TC+TT versus CC)
Discussion

AS is characterized primarily by recurrent inflammatory back pain and bilateral sacroiliitis. Although the multifactorial involvements of AS are well recognized, genetic susceptibility factors are considered to be strong determining factors of this disease. Candidate gene studies need large samples to accomplish sufficient statistical significance and reproducible results. Many genes have been studied in this context, and the PD-1 and CTLA-4 are two popular studied genes of these candidate genes in AS. Nonetheless, some studies on association between PD-1, CTLA-4 polymorphisms and AS risk had used comparatively small samples [17–24], and the results remained conflicting, but not surprising. Therefore, meta-analysis is imperative to ensure adequate statistical power. We conducted this meta-analysis assessing the association between PD-1, CTLA-4 polymorphisms and the susceptibility to AS.

In this meta-analysis, there were eight relevant articles with regard to the association between PD-1, CTLA-4 polymorphisms and the susceptibility to AS. Five studies included 919 AS patients and 983 controls related to PD-1 gene polymorphisms and the susceptibility to AS. There were only three articles including 683 cases and 615 controls related to CTLA-4 gene polymorphisms and AS risk. The present meta-analysis indicated a conspicuously significant relationship between PD-1 rs2227982 gene polymorphism and AS for T allele versus C allele ($P = 0.038$). Because all these eight studies related to AS were also conducted in Asians, we could not conduct the ethnicity-specific meta-analysis. Therefore, more studies are needed to perform in European population.

The PD-1 gene encodes a cell surface membrane protein. The most widely studied PD-1 polymorphisms were the rs11568821, rs2227982 and rs2227981, and in our meta-analysis, we evaluated the associations between rs11568821, rs2227982 and rs2227981 polymorphisms and the susceptibility to AS [17–21]. However, there were two studies related to rs11568821 polymorphism and the risk of AS, and Liu et al. [17] indicated that the GG genotype and the G allele of rs11568821 were identified in all patients and controls. Thus, this research for rs11568821 polymorphism was of no significance and was excluded in the subsequent meta-analysis, and the remaining study could not do meta-analysis. Recently, meta-analysis about the relationships between the PD-1 gene polymorphisms and AS risk was conducted [42, 43]. But our meta-analysis had obviously difference compared with the previous meta-analysis. First, the meta-analysis conducted by Lee et al. [42] was excluded studies that deviated from HWE. Deviation from the HWE among controls could imply potential bias in the selection of controls or genotyping errors. Yet the sensitive analysis of related articles showed that excluding the study with a control group not in HWE, respectively, did not remarkably affect overall results. Thus, our meta-analysis might provide a greater statistical power to make conclusions. Second, compared with the meta-analysis conducted by Lee et al. [42], our meta-analysis assessed...
the associations between rs2227982, rs2227981 polymorphisms and the susceptibility to AS under four genetic models (the allele model, the dominant model, the recessive model and the additive model), which had extraordinary significance in evaluating the relationships. Third, the meta-analysis conducted by Yang et al. [43] was conducted the ethnicity-specific analysis based on Asian and Caucasian populations. Yet the patients of the study conducted by Soleimanifar et al. [19] were Asian group (Iranian patients) and not Caucasian population. Therefore, we could not do ethnicity-specific meta-analysis. The present meta-analysis abandoned the shortcomings of previous studies and revealed that the rs2227982 polymorphism might be a potential risk factor for AS susceptibility, which previously published GWAS studies for AS did not find PD-1 gene might be a potential susceptibility gene for AS. The most impressive discovery among these GWAS studies previously published was the relationship between interleukin-23 receptor (IL-23R) gene polymorphisms and the susceptibility to AS [44].

The CTLA-4 gene is located on a chromosome 2q33, which is critical regulatory molecule expressed in T cells. Although possible associations of the CTLA-4 +49 A/G and −318 C/T polymorphisms with risk of AS were reported, it was still unknown whether there were significant associations between CTLA-4 +49 A/G and −318 C/T polymorphisms and the susceptibility to AS [22–24]. Therefore, we conducted this first analysis about the associations between CTLA-4 +49 A/G and −318 C/T polymorphisms and the risk of AS, but our data did not reveal strong positive results. This was because that the number of studies and the number of subjects in researches selected in this meta-analysis were limited, which might provide insufficient power to estimate the associations between CTLA-4 +49 A/G and −318 C/T polymorphisms and AS risk. Thence, more studies related to the associations between CTLA-4 +49 A/G and −318 C/T polymorphisms and the risk of AS are needed to acquire a more dependable consequence.

It should be noted that the present study had some limitation, which must be considered. Firstly, the number of available articles that related to the associations between PD-1 polymorphisms and the risk of AS was only five, and the number of available studies about the associations between CTLA-4 +49 A/G and −318 C/T polymorphisms and the susceptibility to AS was only three. Thus, the outcomes of this meta-analysis were restricted to a small patient population. It was possible that some connected published studies or unpublished articles with negative conclusions were lost. Therefore, more studies were needed to confirm this consequence and acquire a more dependable result. Secondly, all the included publications were performed in Asians, and we could not do ethnicity-specific meta-analysis. Therefore, future studies should be evaluated in European population and other population. Thirdly, although no evident publication bias was identified, potential bias might have distorted the result of the meta-analysis. Finally, the interaction of susceptibility genes and environment factors led to the pathogenesis of AS, but our study could not valuate gene–gene and gene–environment interactions due to the limited information of selected investigations. In view of these limitations, further research should focus on the associations with gene polymorphisms and clinical or laboratory feature in a large cohort of AS patients.

Despite the above limitations, this systematic analysis of the associations between PD-1, CTLA-4 gene polymorphisms with AS risk was statistically more persuading than any single study. Our study reached a strong conclusion that the minor allele “T” at rs2227982 of PD-1 gene was an independent risk factor for AS, whereas our meta-analysis did not reveal strong positive associations between CTLA-4 +49 A/G and −318 C/T polymorphisms and AS risk. Accordingly, our results supported the fact that the role of PD-1 rs2227982 polymorphism played in the pathogenesis of AS. However, in order to better assess the associations between PD-1, CTLA-4 gene polymorphisms and the susceptibility to AS, further investigations should be conducted in a larger cohort of AS patients. Besides, further investigations are also required to focus on the clinical relevance of these findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The study was approved by the Ethics Committee of the Peking Union Medical College Hospital.

References


rheumatoid arthritis, ankylosing spondylitis, and type 1 diabetes susceptibility. Z Rheumatol 74(3):230–239

