Association of the rs17250932, rs4794067, and rs2240017 polymorphism in the TBX21 gene with autoimmune diseases: A meta-analysis

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Abstract
Objective: To evaluate systematically the association between TBX21 gene polymorphisms (rs17250932, rs2240017, and rs4794067) and the risk of autoimmune diseases in Asian populations.

Methods: The Medline, Web of Science, and Chinese Biomedical Literature Database were used to retrieve eligible studies that were published before July 2020. Pooled odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by using the dominant model, heterozygote contrast model, and allelic contrast model. Publication bias was evaluated using contour-enhanced funnel plots and Egger’s regression test. Sensitivity analysis was conducted to assess the robustness of this meta-analysis.

Results: A total of 12 eligible studies, including 3834 patients and 4824 healthy controls, were recruited in this meta-analysis. The pooled data demonstrated that TBX21 rs2240017 and rs4794067 polymorphisms were significantly associated with the risk of autoimmune diseases in Asian populations in allelic contrast model (OR: 1.456, 95% CI: 1.131–1.875, P = 0.004; OR: 0.766, 95% CI: 0.615–0.954, P = 0.017), heterozygote comparison model (OR: 1.647, 95% CI: 1.239–2.189, P = 0.001; OR: 0.796, 95% CI: 0.634–0.999, P = 0.049), and dominant model (OR: 1.572, 95% CI: 1.194–2.071, P = 0.004; OR: 0.767, 95% CI: 0.607–0.970, P = 0.027). The G allele of rs2240017 may be a risk factor for autoimmune diseases, and the T allele of rs4794067 may increase the risk of autoimmune diseases. However, we failed to find evidence of the association between TBX21 rs17250932 polymorphism and susceptibility to autoimmune diseases. No publication bias was established in this meta-analysis.

Conclusion: This meta-analysis indicated that TBX21 rs2240017 and rs4794067 polymorphism confer susceptibility to autoimmune diseases, but not rs17250932.

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Introduction

Autoimmune diseases are a kind of chronic complex diseases characterized by loss of autoimmune tolerance; 7.6%–9.4% of general population suffers from autoimmune diseases, and the incidences are still increasing. Autoimmune diseases are now ranked within the top 10 causes of death, resulting in a great economic burden. Although the mechanism of autoimmune diseases is still unclear, the combination of genetic predisposition and environmental factors is thought to play an important role in the etiology of these diseases. Data from human genome-wide association studies (GWAS), linkage, and association studies indicate that autoimmune diseases may share common genetic background.

T cell-specific T-box transcription factor (TBX21) gene is a member of a phylogenetically conserved family of genes that share a common DNA-binding domain, the T-box, and is located on human chromosome 17q21.32. The transcription factor T-bet, encoded by TBX21 gene, has recently emerged as a central player in autoimmune diseases. As a key regulator of Th1 cell differentiation, T-bet can initiate naive Th precursor cells developed to Th1 lineage and play an essential role in promoting IFN-γ production. For example, T-bet transgenic mice avoided developing collagen-induced arthritis, and another mouse model showed an ameliorated Type 1 diabetes in the absence of T-bet. The systemic lupus erythematosus (SLE) was also established to be less susceptibility in T-bet deficient mice. Previous studies have shown that TBX21 was a strong candidate gene because of its role in Th1/Th2 balance. Collectively, these findings suggest that TBX21 may play a critical role in multiple immune-mediated diseases.

A number of published case-control studies have been conducted to evaluate the association between TBX21 rs17250932, rs4794067, and rs2240017 polymorphisms and autoimmune diseases, including SLE, rheumatoid arthritis (RA), type 1 diabetes (T1D), Behcet’s disease, and autoimmune hepatitis (A1H). The TBX21 gene, has recently emerged as a central player in autoimmune diseases.

Methods and materials

Search strategy

A well-conducted search was performed by two investigators independently to retrieve all the literature examining the association between TBX21 single nucleotide polymorphisms (SNPs; rs17250932, rs4794067, and rs2240017) and autoimmune diseases. The authors used three bibliographic databases (Medline, Web of Science, and Chinese Biomedical Literature Database) to retrieve eligible studies. The combination of keywords, such as “TBX21,” “T-bet,” “polymorphism,” “autoimmune disease,” and the names of individual diseases, served as Medical Subject Heading (MeSH) terms and/or text words. Additional studies were supplemented by the references of relevant original research reports and related articles.

Inclusion and exclusion criteria

We made the following restrictions for the retrieved literatures: (1) unrelated case-control or cohort design; (2) evaluating the association between TBX21 polymorphism and autoimmune diseases; (3) containing available and sufficient data for comparison and calculating odds ratios (OR) and 95% confidence intervals (CI). Duplicate datasets or studies containing family members were excluded. In addition, autoimmune diseases should be diagnosed according to the respective classification criteria.

Data extraction

First author, year of publication, country and ethnicity of the studied population, demographic information, number of cases and controls, and the allele and genotype frequency of rs17250932, rs4794067, and rs2240017 were extracted from each literature by two authors independently. When the two authors were at odds, the third author was consulted. The studies which contained more than one disease or SNP were treated as separate studies.

Evaluation of statistic association

The meta-analysis aimed to evaluate the strength of association between TBX21 rs17250932, rs4794067, and rs2240017 polymorphisms and autoimmune diseases by calculating the total OR and 95% CI. Three genetic models were performed, including dominant model, heterozygote contrast model, and allelic contrast model. The χ² goodness-of-fit tests were used to examine the existence of the Hardy-Weinberg equilibrium (HWE); P < 0.05 was considered to be statistically significant.

Heterogeneity was assessed with Cochran’s Q test and I² statistic. P < 0.10 indicated a significant Q statistic and the existence of within- and between-study variation I² statistic values from 0 to 100%; 25%, 50%, and 75% represented a low, moderate, and high heterogeneity, respectively. The pooled OR values and their 95% CIs were obtained by using random effect model in case of significant heterogeneity (P < 0.10 or I² > 50%), otherwise a fixed effect model (P < 0.10 or I² > 50%) was used in this meta-analysis.

Contour-enhanced funnel plots were carried out to evaluate the potential publication bias intuitively. The Beggs’s and Egger’s tests were also performed to quantify the evidence for asymmetry and P < 0.05 was considered to be a statistically significant representation of publication bias. By removing one study each time and repeating the analysis, sensitivity analysis was performed to value the stability of this meta-analysis. All statistical analyses were carried out by STATA 12.0 software (Stata Corp, College Station, TX, USA).
Results

Characteristics of eligible studies

In all, 73 studies were retrieved until July 31, 2020 after a systematical research was done in the above-mentioned bibliographic databases. Among these articles, 20 duplicate articles were excluded; 20 studies were excluded for not mentioning SNPs and autoimmune diseases in the title and on abstract screening. Four animal experimental studies, one non-case control study, and seven reviews were also excluded. After excluding three articles that mainly investigated irrelevant SNPs and six articles whose raw data were unavailable, 12 articles met the inclusion criteria. However, after a careful examination, we determined that two articles should be excluded because one of them was a pedigree study and the other deviated from HWE. Finally, 10 articles were identified as eligible studies. It is worth noting that one article only provided the frequency of rs17250932 T/C allele in the controls but did not provided data on TT/TC/CC genotypes. Therefore, in this article we included only rs4794067 and rs2240017 for meta-analysis. Of these 10 articles, two articles investigated two types of autoimmune diseases, respectively, so each autoimmune disease was considered to be a separate study. Therefore, 12 eligible studies, including 3834 patients and 4824 healthy controls, were recruited in this meta-analysis. The literature selection process is shown in Figure 1.

The association of TBX21 rs17250932 polymorphism and autoimmune disease susceptibility

The findings indicated that no relationship existed between rs17259032 polymorphism and autoimmune diseases in the above-mentioned three genetic contrast models, and the pooled OR (95% CI) was 0.842 (0.619–1.144) in allelic contrast model, 0.832 (0.580–1.195) in heterozygote comparison, and 0.830 (0.588–1.171) in dominant model (all P-values >0.05). The results are summarized in Table 2 and Figure 2.

The association of TBX21 rs2240017 polymorphism and autoimmune disease susceptibility

A significant association between TBX21 rs2240017 polymorphism and autoimmune diseases was revealed in allelic contrast model (OR: 1.456, 95% CI: 1.131–1.875, P = 0.004), heterozygote comparison model (OR: 1.647, 95% CI: 1.239–2.189, P = 0.001), and dominant mode (OR: 1.572, 95% CI: 1.194–2.071, P = 0.004) in Asian populations. A summary of meta-analysis findings regarding the association of TBX21 rs2240017 polymorphism and autoimmune disease susceptibility is provided in Table 2 and Figure 2.

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**Figure 1** Flowchart for identification of studies in the meta-analysis.
Table 1  Characteristics of individual studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>SNP</th>
<th>First author</th>
<th>Year</th>
<th>Disease</th>
<th>Country</th>
<th>Genotyping methods</th>
<th>Sample size (Case/control)</th>
<th>Genotypes (Case/control)</th>
<th>Allele (Case/control)</th>
<th>HWE</th>
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<td>You Y</td>
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<td>SLE</td>
<td>China</td>
<td>PCR–RFLP</td>
<td>248/261</td>
<td>226/210, 20/50, 2/1</td>
<td>472/470, 24/52</td>
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<td></td>
<td>Chae SC</td>
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<td>RA</td>
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<td>367/572</td>
<td>332/546, 33/26, 0/0</td>
<td>697/1118, 33/26</td>
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<td>Chen S</td>
<td>2011</td>
<td>AIH</td>
<td>China</td>
<td>PCR–RFLP</td>
<td>84/318</td>
<td>72/267, 12/50, 0/1</td>
<td>156/584, 12/52</td>
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<td>HD</td>
<td>Japan</td>
<td>PCR</td>
<td>90/79</td>
<td>58/60, 3/9, 0/0</td>
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<td>China</td>
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<td>275/261</td>
<td>241/224, 34/37, 0/0</td>
<td>516/485, 34/37</td>
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<td>Liao D</td>
<td>2015</td>
<td>BD</td>
<td>China</td>
<td>PCR–RFLP</td>
<td>401/613</td>
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<td>VKH</td>
<td>China</td>
<td>PCR–RFLP</td>
<td>401/613</td>
<td>347/508, 50/78, 2/6</td>
<td>744/1094, 54/90</td>
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<td>2012</td>
<td>AA</td>
<td>China</td>
<td>PCR–RFLP</td>
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<td>177/167, 25/28, 0/0</td>
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<td>T1D</td>
<td>Japan</td>
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<td>RS</td>
<td>Japan</td>
<td>PCR</td>
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<td>25/35, 3/6, 1/3</td>
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<td>rs4794067</td>
<td>You Y</td>
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<td>SLE</td>
<td>China</td>
<td>PCR–RFLP</td>
<td>248/261</td>
<td>202/192, 43/63, 3/6</td>
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<td>Sequenom (MassArray)</td>
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<td>Japan</td>
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<td>57/43, 13/14, 0/3</td>
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<td>GD</td>
<td>Japan</td>
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<td>75/43, 21/14, 1/3</td>
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<td>Zhang D</td>
<td>2014</td>
<td>ITP</td>
<td>China</td>
<td>PCR–RFLP</td>
<td>275/261</td>
<td>228/193, 46/66, 1/2</td>
<td>502/452, 48/70</td>
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<td>2013</td>
<td>RS</td>
<td>Japan</td>
<td>PCR</td>
<td>29/113</td>
<td>23/96, 6/16, 0/1</td>
<td>52/208, 6/18</td>
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<td>2015</td>
<td>VKH</td>
<td>China</td>
<td>PCR–RFLP</td>
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<td>319/464, 75/116, 6/13</td>
<td>713/1044, 87/142</td>
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<td>Ge ML</td>
<td>2012</td>
<td>AA</td>
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<td>PCR–RFLP</td>
<td>202/195</td>
<td>172/149, 30/46, 0/0</td>
<td>374/344, 30/46</td>
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Note: For rs17250932 and rs4794067, MM, Mm and mm stand for TT, TC and CC genotypes, respectively. For rs2240017, MM, Mm and mm stand for CC, CG and GG genotypes, respectively.
The association of TBX21 rs4794067 polymorphism and autoimmune disease susceptibility

A significant association was found between TBX21 rs4794067 polymorphism and autoimmune diseases using allelic contrast model (OR: 0.766, 95% CI: 0.615–0.954, \( P = 0.017 \)), heterozygote comparison model (OR: 0.796, 95% CI: 0.634–0.999, \( P = 0.049 \)), and dominant model (OR: 0.767, 95% CI: 0.607–0.970, \( P = 0.027 \)) in Asian populations. However, it should be noted that the marginal value was approximately equal to 1 in the heterozygote comparison model. Meta-analysis results concerning the association of TBX21 rs4794067 polymorphism and autoimmune disease susceptibility is given in Table 2 and Figure 2.

Test of heterogeneity

As shown in Table 2, heterogeneity was found when analyzing the TBX21 polymorphisms rs17250932 and rs4794067 and autoimmune diseases in allelic contrast model, heterozygote comparison model, and dominant model (all \( P \)-values <0.05), but no heterogeneity was found in rs2240017 in the three genetic models (all \( P \)-values >0.05). Subgroup analyses and meta-regression were conducted to explore the potential sources of heterogeneity. The results showed that the year of publication and genotyping methods were not able to explain heterogeneity for rs17250932 (all \( P \)-values >0.05), and the genotyping methods and country were not statistically correlated with heterogeneity for rs4794067. However, we established that South Korea could be responsible for heterogeneity for rs17250932, and the year of publication may statistically correlate with heterogeneity for rs4794067.

Publication bias

Contour-enhanced funnel plots and the Begg's and Egger's tests were carried out to evaluate the potential publication bias in this meta-analysis. The results revealed that nonstatistically significant asymmetry was found in the Asian populations for rs17250932 (Egger's test: \( t = 0.383, \ P = 0.232 > 0.05 \); Begg's test: \( z = 1.15; \ P = 0.251 > 0.05 \)), and for rs2240017 (Egger's test: \( t = 0.220, \ P = 0.359 > 0.05 \); Begg's test: \( z = 0.00; \ P = 1.000 > 0.05 \)). There seems to be no significant asymmetry in the Asian populations for rs4794067 based on the intuitive observation of the contour-enhanced funnel plots, and the results of the Berger's test also supported that no significant publication bias was found (Begg's test: \( z = 1.25; \ P = 0.210 > 0.05 \); however, a statistically significant asymmetry was found in Egger's test (Egger's test: \( t = −2.90, \ P = 0.020 < 0.05 \)). Therefore, we performed trim and fill funnel plot for further verification (data not shown), and the results showed that there was no evidence of missing studies, thus confirming the absence of publication bias (Figure 3).

Sensitivity analysis

After the single study was excluded, nonstatistically significant change was observed in the recalculated pooled ORs.
in the Asian populations for rs4794067 in allelic contrast model, indicating that the results of this meta-analysis were stable. For rs17250932, after excluding the study done by Chae et al.,\textsuperscript{14} a marginal statistically significant change ($P = 0.046$) was shown and the pooled OR (95% CI) changed from 0.842 (0.619–1.144) to 0.772 (0.599–0.995) by using allelic contrast model. Since only three articles about rs2240017 were included in the meta-analysis, we did not include them in sensitivity analysis (Figure 4).

Discussion

Autoimmune diseases are a group of diseases with similar pathogenesis and characterized by abnormal immunity and tissue destruction.\textsuperscript{23} Although there are many hypotheses about the pathogenesis of autoimmune diseases, increasingly data have shown that the interaction of environmental and genetic factors may have an important effect on the occurrence and development of autoimmune diseases.\textsuperscript{24} As one of the most common genetic variations in human genome, SNP may play an important role in autoimmune diseases.\textsuperscript{25} Previous studies on genetic association have shown that autoimmune diseases may share susceptibility genes.\textsuperscript{26}

T-bet, encoded by the TBX21 gene, is an important Th1 transcription factor closely related to the occurrence and treatment of autoimmune diseases.\textsuperscript{27} More specifically, two important polymorphisms rs17250932 and rs4794067 in the promoter region of the TBX21 gene and rs2240017 in the nonpromoter region are considered to be one of the possible genetic risk factors for autoimmune diseases, and many studies have reported that the above SNPs may be associated with genetic susceptibility to autoimmune diseases in Asian populations. However, the results of some studies could be contradictory. For example, You et al. found that rs17250932 T allele was a risk factor for SLE,\textsuperscript{13} while Chae et al. showed that rs17250932C allele was a risk factor for RA,\textsuperscript{14} but Leng et al. failed to find a relationship between rs17250932 and SLE.\textsuperscript{12} Therefore, in order to comprehensively evaluate the relationship between TBX21 rs17250932, rs2240017, and rs4794067 polymorphisms and the risk of autoimmune diseases, we conducted this meta-analysis. To the best of our knowledge, this is the first meta-analysis to evaluate the relationship between TBX21 gene polymorphism and genetic susceptibility to autoimmune diseases, and is more effective than any previous case-control study.

In this meta-analysis, we demonstrated that TBX21 rs2240017 and rs4794067 polymorphisms were significantly associated with the risk of autoimmune diseases in Asian populations. The results demonstrated that the G allele of rs2240017 may be a risk factor for autoimmune diseases, and the T allele of rs4794067 may increase the risk of autoimmune diseases. However, we failed to find evidence of the association between TBX21 rs17250932 polymorphism and susceptibility to autoimmune diseases. T-bet is considered to be the main regulator of CD4+ Th1 cell differentiation and can promote the production of IFN-γ by Th1 cells.
and NK cells. At the same time, T-bet also has a regulatory effect on IgG2a produced by B cells, although this effect may be based on the action of IFN-γ. These function of T-bet are considered to affect the process of autoimmunity. Both rs17250932 and rs4794067 are located in the promoter region of \( TBX21 \). These SNP variants may be related to the translation of mRNA, thus regulating the expression or function of T-bet. Therefore, the polymorphism of \( TBX21 \) gene may change the expression level of mRNA, resulting in changes in protein expression or the production of autoantibodies or other immune diseases. All these make \( TBX21 \) an interesting candidate for autoimmune diseases. However, the future studies could confirm this hypothesis. It is worth mentioning that there is heterogeneity in our study. In the meta-analysis of rs17250932, we found strong heterogeneity, but after excluding the study done by Chae et al., the heterogeneity disappeared and there was a significant association between rs17250932 and autoimmune diseases, and the \( T \) allele was the potential risk factor. However, after a careful review of Chae et al., we did not find any difference between this and other articles, so we concluded that rs17250932 polymorphism has nothing to do with autoimmune diseases, and more related studies need to be included to confirm this view.

However, because of the possible limitations of this meta-analysis, it is necessary to be cautious in drawing conclusions based on this study. First, autoimmune disease is a complex illness caused by the interaction of genetic and environmental factors. The current polymorphism may affect the occurrence of autoimmune diseases to some extent, which is difficult to be detected by meta-analysis. Second, the article retrieval in this meta-analysis was limited to three electronic databases (Medline, Web of Science, and Chinese Biomedical Literature Database), and the search languages were limited to English and Chinese. Owing to the limitations of search databases and languages, there could be a possibility of publication bias. Third, all the original studies were based on Asian populations. Although two articles reported the association between rs17250932 and rs2240017 and autoimmune diseases in the Caucasian population, neither of them was included in this study because one of them was a family study and the other did not conform to HWE. Fourth, the number of studies included in this meta-analysis was small, and lacked sufficient research to carry out subgroup analysis according to the type of disease. Fifth, significant heterogeneity was found in the analysis for rs17250932 and rs4794067 by using three genetic models.

According to the results of subgroup analyses and meta-regression, the year of publication were considered to be the main sources of rs4794067 heterogeneity, while South Korea could be responsible for heterogeneity for rs17250932. Sixth, owing to data limitations or the unavailability of data, this meta-analysis did not take into account the impact of gender, age, and other environmental factors on autoimmune diseases.

Overall, results of our meta-analysis showed that \( TBX21 \) rs2240017 and rs4794067 polymorphisms may be associated with susceptibility to autoimmune diseases in Asian populations, but we failed to found an association between rs17250932 and autoimmune diseases. In Asian populations, the \( T \) allele of rs4794067 in the promoter region may be associated with increased susceptibility to autoimmune diseases, while the \( G \) allele of rs2240017 is shown to be a potential risk factor. However, this study has some limitations in exploring the exact mechanism of \( TBX21 \) gene polymorphism affecting the susceptibility to autoimmune diseases. Therefore, further studies, including larger sample sizes and well-designed case controls in different ethnic groups, are needed to reveal the exact role of \( TBX21 \) SNPs in the pathogenesis of autoimmune diseases.

Acknowledgments

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Conflict of interest

Authors declare that they have no conflict of interest.

Author contributions

Hai-li Wang and Hong Wang: literature search, manuscript preparation, statistical analysis. Yue Wu, Hua-yun Ling and
14. Chae SC, Shim SC, Chung HT. Association of


References

