No association of CTLA-4 polymorphisms with susceptibility to Behcet disease

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ABSTRACT

Background: Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is a key negative regulator of T lymphocytes and has been shown to be associated with a number of autoimmune diseases. The present study was performed to assess the association between CTLA-4 polymorphisms and Behcet disease (BD) in Chinese patients.

Methods: Two hundred and twenty-eight BD patients and 207 controls were analysed for four single nucleotide polymorphisms (SNPs) (−1661A/G, −318C/T, +49G/A and CT60G/A) in the CTLA-4 gene by PCR-restriction fragment length polymorphism (RFLP) analysis. The association between SNP +49A/G and BD in Chinese population as well as other ethnic groups was analysed by meta-analysis.

Results: No association could be detected between CTLA-4 SNPs or haplotypes and BD. Also, no association was observed between CTLA-4 polymorphisms and BD subgroups, stratified by clinical features. A meta-analysis showed that there was no heterogeneity between studies (p = 0.60, I² = 0%) and that CTLA-4 SNP +49 was not associated with BD (overall effect: Z = 0.26, p = 0.79).

Conclusion: This study and a meta-analysis failed to demonstrate any association between the tested CTLA-4 polymorphisms and BD.
and stained with GoldView (SBS Genetech, Beijing) (fig 1). Appropriate controls (no template and known genotype) were included in each typing run.

**STATISTICAL ANALYSIS**

SPSS software (version 10.0; SPSS, Chicago) was used to analyse the data. The software Haploview 3.32 was used for testing pairwise linkage disequilibrium and to estimate the haplotype frequency.16 The default setting, confidence interval algorithm, was applied to our analysis. The allele and genotype distributions of SNPs in CTLA-4 were compared between patients and controls by $\chi^2$ test and Fisher exact correction. Allele and genotype distributions were also analysed between patients with or without certain clinical features by $\chi^2$ test. A p value of <0.05 was considered significant. A meta-analysis was performed using the Review Manager software package (version 4.2) (http://www.cc-ims.net/RevMan).

**RESULTS**

**Allele and genotype frequencies of CTLA-4**

All samples from 228 BD patients and 207 controls were genotyped for four SNPs in the CTLA-4 gene. The distribution of genotype frequencies of each SNP in our cohort was in Hardy–Weinberg equilibrium. Table 2 summarises the results from our study. There were no differences in the allele or genotype frequencies of the four SNPs between BD patients and controls. Furthermore, no association was observed when BD patients were subdivided according to sex, extraocular clinical features and HLA-B51 (data not shown).

**Haplotype frequencies of CTLA-4**

Haplotypes were calculated with the program Haploview 3.32. Four SNPs were in tight linkage disequilibrium with each other. Three haplotypes were obtained which have a frequency of more than 3% in the patient or control group (table 3). As reported previously,15 the haplotype $-1661A:\!-318C:\!:+49G$: $CT60G$ was the most prevalent haplotype. There were no differences in haplotype frequencies between the patients and the controls (table 3). Stratification of patients by sex, extraocular manifestations and HLA-B51 did not reveal any correlation between these three haplotypes and subgroups of patients (data not shown).

**Meta-analysis of CTLA-4 polymorphism**

For a meta-analysis, we searched the Medline database and checked the reference lists of the retrieved articles for all studies which tested the association between CTLA-4 polymorphism and BD patients. Three studies were found and included in this meta-analysis. Two studies were from Turkey, and one study included Caucasian patients (table 4). The association between SNP +49 and BD patients was assessed using the data from these three studies as well as the present study. Genotype frequencies of SNP +49 in these four cohorts were in Hardy–Weinberg Equilibrium, and there was no heterogeneity among these studies (p = 0.60, I² = 0%). The pooled OR was 0.98 (0.83 to 1.15) for fixed effects. The results showed no association between CTLA-4 SNP +49 and BD (overall effect: $Z = 0.26$, p = 0.79).

**DISCUSSION**

In this study, we examined the association between CTLA-4 polymorphisms and BD in a Chinese Han population. Our results showed no association between CTLA-4 SNPs and BD or between haplotypes and BD. There was also no association between CTLA-4 polymorphisms and BD after stratification by clinical features. Meta-analysis using data from four studies also showed no association between CTLA-4 SNP +49 and BD.
All four SNPs assessed in our study have been previously shown to be associated with a variety of autoimmune diseases.\(^8\)\(^{17}\)\(^{18}\) Sallakci \textit{et al}\(^{17}\) showed that the +49A allele and the AA genotype were significantly higher in BD patients with ocular or skin involvement. They did not find any association when comparing all BD patients with controls and concluded that CTLA-4 is rather a disease modifying than a susceptibility gene. Studies by Gunesacar \textit{et al}\(^{18}\) and Bye \textit{et al}\(^{18}\) however failed to demonstrate any correlation of CTLA-4 polymorphisms with BD subgroup that were classified according to clinical features. Our study also did not support the association between CTLA-4 polymorphisms and BD subgroup stratification by clinical features. The observed discrepancy between these studies may result from different genetic backgrounds in the populations analysed or from sampling error. Meta-analysis has been used to assess the association between gene polymorphisms and systemic lupus diseases in different races. Certain studies showed an association between CTLA-4 gene polymorphisms and BD subgroup stratification by clinical features. The observed discrepancy between these studies may result from different genetic backgrounds in the populations analysed or from sampling error. Meta-analysis has been used to demonstrate any correlation of CTLA-4 polymorphisms with autoimmune disease mediated by T cells.\(^{15}\) Unexpectedly, however, no association was found between CTLA-4 polymorphisms and BD, another frequent uveitis in China. The Th1/Th2 paradigm varies significantly in different diseases. CTLA-4 has been regarded as a genetic master switch for autoimmunity and plays a critical role in the Th1/Th2 balance. Therefore, the different associations between CTLA-4 SNP +49 and autoimmune diseases may lead to the varied Th1/Th2 paradigms in different diseases.

In a recent study, we showed a significant association of the CTLA-4 haplotype −1661A:−318C:+49G:CT60G and the G allele at SNP +49 with the susceptibility to VKH syndrome, another common uveitis entity in China which is considered an autoimmune disease mediated by T cells.\(^{15}\) Unexpectedly, however, no association was found between CTLA-4 polymorphisms and BD, another frequent uveitis in China. The conflicting results may be explained by the different nature of these two common autoimmune uveitis entities. VKH syndrome is a granulomatous inflammation, while BD is in fact a non-granulomatous inflammation. In addition, although Behçet disease is considered as an autoimmune disease mediated by T cells, the observed discrepancy between our study and others may result from different genetic backgrounds in the populations analysed or from sampling error. Meta-analysis has been used to assess the association between gene polymorphisms and diseases in different races. Certain studies showed an association between CTLA-4 gene polymorphisms and systemic lupus erythematosus (SLE).\(^{19}\) While other studies did not support these results.\(^{20}\) Meta-analysis using the data from a variety of studies showed that there was an association between CTLA-4 gene polymorphism and SLE.\(^{21}\) We therefore performed a meta-analysis using the data available in the literature\(^{15}\)\(^{15}\) and those presented in our study. The results showed that there was no heterogeneity among the four studies and that CTLA-4 SNP +49 was not associated with BD. This difference in the results concerning association between CTLA-4 SNP +49 and certain autoimmune diseases may result from the different pathogenesis of these diseases. The Th1/Th2 paradigm varies significantly in different diseases. CTLA-4 has been regarded as a genetic master switch for autoimmunity and plays a critical role in the Th1/Th2 balance. Therefore, the different associations between CTLA-4 SNP +49 and autoimmune diseases may lead to the varied Th1/Th2 paradigms in different diseases.

### Table 2

| Allele and genotype frequencies of cytotoxic T lymphocyte-associated antigen 4 polymorphisms in Han Chinese Behçet disease patients and controls |
|-----------------|-----------------|----------------|----------------|----------------|
| Alleles and genotypes | Behçet disease (%) | Controls no (%) | p Value | Odds ratio (95% CI) |
| −1661A/G | A 388 (85.8) | 352 (85.9) | 0.996 | 0.999 (0.881 to 1.146) |
| | G 64 (14.2) | 58 (14.1) | 1 |  |
| AA | 165 (73.0) | 149 (72.7) | 0.935 | 1.031 (0.669 to 1.588) |
| AG | 58 (25.7) | 54 (26.3) | 1 |  |
| GG | 3 (1.3) | 2 (1.0) | 1 |  |
| −318C/T | C 390 (86.3) | 352 (85.4) | 0.721 | 1.072 (0.731 to 1.573) |
| | T 62 (13.7) | 60 (14.6) | 1 |  |
| CC | 166 (73.4) | 149 (72.3) | 0.846 | 1.071 (0.731 to 1.573) |
| CT | 58 (25.7) | 54 (26.2) | 1 |  |
| +49G/A | TT 2 (0.9) | 3 (1.5) | 0.645 | 0.104 (0.299) |
| | A 152 (33.3) | 149 (26.2) | 0.381 | 0.883 (0.657 to 1.168) |
| | G 304 (66.7) | 263 (36.8) | 1 |  |
| AA | 20 (8.8) | 26 (12.6) | 0.429 | 0.666 (0.350 to 1.268) |
| GA | 112 (49.1) | 97 (47.1) | 1 |  |
| GG | 96 (42.1) | 83 (36.0) | 1.002 (0.671 to 1.495) |
| CT60G/A | A 99 (21.7) | 92 (22.2) | 0.856 | 0.971 (0.704 to 1.338) |
| | G 357 (78.3) | 322 (77.8) | 1 |  |
| AA | 9 (3.9) | 11 (5.3) | 0.766 | 0.707 (0.277 to 1.805) |
| GA | 81 (35.5) | 70 (33.8) | 1 |  |
| GG | 138 (60.6) | 126 (60.9) | 0.947 (0.634 to 1.413) |

Allele and genotypes frequencies were compared between Behçet disease and control by χ² test. Single nucleotide polymorphism (SNP) −1661A/G was not identified successfully in four samples; SNP −318C/T was not identified successfully in three samples; and SNP +49G/A was not identified successfully in one sample.

### Table 3

<table>
<thead>
<tr>
<th>Haplotypes*</th>
<th>Behçet disease (%)†</th>
<th>Controls (%)†</th>
<th>χ²‡</th>
<th>p Value‡</th>
<th>OR (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACGG</td>
<td>288.7 (63.3)</td>
<td>252.1 (60.9)</td>
<td>0.580</td>
<td>0.446</td>
<td>1.11 (0.846 to 1.464)</td>
</tr>
<tr>
<td>ACAA</td>
<td>85.3 (18.6)</td>
<td>80.3 (19.4)</td>
<td>0.066</td>
<td>0.797</td>
<td>0.96 (0.681 to 1.343)</td>
</tr>
<tr>
<td>GTAG</td>
<td>55.3 (12.1)</td>
<td>53.9 (13.0)</td>
<td>0.191</td>
<td>0.662</td>
<td>0.91 (0.612 to 1.366)</td>
</tr>
</tbody>
</table>

Haplotypes with a frequency less than 3% are not listed.
*Haplotypes were constructed from single nucleotide polymorphisms in the order of −1661A/G, −318C/T, +49G/A and CT60G/A using the Haploview 3.32 software based on an accelerated EM algorithm.
†Haplotypes numbers and frequencies were estimated using the Haploview 3.32 software.
‡The percentages of Behçet disease and controls with a certain haplotype were compared with the percentages of Behçet disease and controls without this kind haplotype by the χ² test (2×2 table).
cells, it is still proposed that it may represent an autoinflammatory condition developing in the background of enhanced innate immune reactivity.22 23

In conclusion, all results in our study failed to demonstrate any association of tested CTLA-4 gene polymorphisms with BD in Chinese patients.

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