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Association of *LACC1*, *CEBPB-PTPN1*, *RIPK2* and *ADO-EGR2* with ocular Behcet's disease in a Chinese Han population

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ABSTRACT

Background An Immunochip study recently identified the association of a number of new genetic loci with Behcet's disease (BD).

Objective To confirm the association between new genetic loci reported in an Immunochip study and BD in a Han Chinese population.

Methods A two-stage association study was carried out in 1238 patients with BD and 1458 healthy controls. Twenty-two candidate single nucleotide polymorphisms (SNPs) were selected for genotyping by iPLEXGold genotyping or TaqMan SNP assays and a meta-analysis was performed for significantly associated markers.

Results The results showed that four SNPs (*LACC1*/rs9316059, *CEBPB-PTPN1*/rs913678, *ADO-EGR2*/rs224127 and *RIPK2*/rs10094579) were associated with BD in an allelic association test (rs9316059 T allele: $p_c=4.95 \times 10^{-8}$, OR=0.687; rs913678 C allele: $p_c=3.01 \times 10^{-4}$, OR=1.297; rs224127 A allele: $p_c=3.77 \times 10^{-4}$, OR=1.274; rs10094579 A allele: $p_c=6.93 \times 10^{-4}$, OR=1.302). For four SNPs tested by meta-analysis, the association with BD was strengthened and all exceeded genome-wide significance (rs9316059: $p=2.96 \times 10^{-16}$; rs913678: $p=2.09 \times 10^{-16}$; rs224127: $p=5.28 \times 10^{-13}$; rs10094579: $p=9.21 \times 10^{-11}$).

Conclusions Our findings confirmed the association of four loci (*LACC1*, *CEBPB-PTPN1*, *ADO-EGR2* and *RIPK2*) in Chinese Han patients with BD.

INTRODUCTION

BD is a chronic systemic vasculitis that mainly presents with recurrent uveitis, oral ulcers, genital ulcers and multiple skin lesions.¹ BD is more common among countries along the 'silk route' from the Mediterranean, Middle East, China and Japan, but is rare in the USA and Europe.² Although the aetiology and pathogenesis of BD remain unclear, it is currently thought that both genetic and environmental factors contribute to disease occurrence and development. In addition to *HLA-B*51* which has been shown to have the strongest association with BD,^{3–7} a series of genome-wide association studies in different populations have identified a number of non-human leucocyte antigen susceptibility loci for BD, including *IL23R-IL12RB2*, *IL10*, *STAT4*, *CCR1-CCR3*, *KLRC4*, *ERAP1*, *TNFAIP3*, *IL12A* and *FUT2*.^{3–11} These findings have increased our understanding of immunogenetic factors involved in the disease. However, these

identified genetic risk loci do not fully explain the genetic aetiology of BD, and other genetic factors remain to be identified.

Recently, Takeuchi *et al*¹² conducted a genetic association study using the Immunochip genotyping array in a Turkish cohort (1900 patients with BD and 1779 controls) and further confirmed the associations of BD with *HLA-B*51*, *IL23R-IL12RB2*, *IL10*, *CCR1*, *KLRC4*, *ERAP1*, *IL12A* and *FUT2*. More significantly, the same study identified six new BD risk loci (*IL1A-IL1B*, *IRF8*, *CEBPB-PTPN1*, *ADO-EGR2*, *RIPK2* and *LACC1*) with genome-wide significance ($p < 5 \times 10^{-8}$) and a number of new loci with a suggestive disease association ($p < 5 \times 10^{-5}$). Takeuchi *et al* also performed a replication study¹² and confirmed the association for some, but not all, loci in Iranian and Japanese cohorts. These findings indicated that the genetic background of BD may differ among different ethnic groups. Since the novel loci mentioned above have not yet been tested in other populations, we decided to perform a replication study to assess whether the findings of the Takeuchi study could be confirmed in Chinese Han patients with BD.

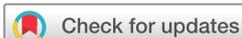
MATERIALS AND METHODS

Subjects

A total of 1238 patients with BD and 1458 healthy controls were included in the present study. All the patients were Han Chinese and recruited from the Department of Ophthalmology of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) from June 2008 to December 2016. BD was strictly diagnosed based on the criteria of the International Study Group for BD¹³ and all patients had uveitis. All control subjects were matched for age, sex, ethnicity (Han Chinese) and geographic origin with patients with BD. The present study was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participating individuals.

SNPs selection

We selected 27 candidate SNPs in 20 susceptibility loci from the BD Immunochip association study.¹² Criteria used were as follows: we selected the lead SNPs in the potential susceptibility loci with a p value less than 5×10^{-5} . In order to increase the reliability of our results, the other



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Table 1 The potential susceptibility loci identified in the 'ImmunoChip' study for Behcet's disease

| Number | SNP | Nearest gene(s) | Chromosome | Reported p values* |
|--------|--------------|---------------------|------------|--------------------|
| 1 | rs17753641† | <i>IL12A</i> | 3 | 8.11E-10 |
| 2 | rs17810546† | <i>IL12A</i> | 3 | 1.01E-07 |
| 3 | rs601338‡ | <i>FUT2</i> | 19 | 6.51E-09 |
| 4 | rs1047781‡ | <i>FUT2</i> | 19 | 6.50E-04 |
| 5 | rs3783550 | <i>IL1A-IL1B</i> | 2 | 1.29E-08 |
| 6 | rs913678 | <i>CEBPB-PTPN1</i> | 20 | 1.10E-09 |
| 7 | rs7075773§ | <i>ADO-EGR2</i> | 10 | 1.69E-09 |
| 8 | rs1509966‡ | <i>ADO-EGR2</i> | 10 | 1.47E-06 |
| 9 | rs224127‡ | <i>ADO-EGR2</i> | 10 | 1.56E-06 |
| 10 | rs9316059‡ | <i>LACC1</i> | 13 | 1.16E-05 |
| 11 | rs10176241 | <i>THADA</i> | 2 | 3.05E-05 |
| 12 | rs79891766† | <i>LONRF2</i> | 2 | 3.60E-05 |
| 13 | rs116379815† | <i>RBM6</i> | 3 | 4.17E-07 |
| 14 | rs11248047 | <i>CPLX1</i> | 4 | 1.27E-07 |
| 15 | rs13190001† | <i>C5orf56</i> | 5 | 1.19E-05 |
| 16 | rs17705333 | <i>INHBA</i> | 7 | 1.82E-05 |
| 17 | rs9656588 | <i>IKZF1</i> | 7 | 5.28E-06 |
| 18 | rs10094579 | <i>RIPK2</i> | 8 | 6.03E-07 |
| 19 | rs2230801‡ | <i>RIPK2</i> | 8 | 9.60E-06 |
| 20 | rs911603 | <i>TNFSF8</i> | 9 | 1.17E-05 |
| 21 | rs28734985 | <i>IPMK-UBE2D1</i> | 10 | 4.10E-05 |
| 22 | rs1698386§† | <i>IPMK-UBE2D1</i> | 10 | 1.36E-05 |
| 23 | rs10896027 | <i>MAP3K11-RELA</i> | 11 | 2.58E-05 |
| 24 | rs58950470§ | <i>MAP3K11-RELA</i> | 11 | 6.25E-07 |
| 25 | rs4906762 | <i>ATP10A</i> | 15 | 3.81E-05 |
| 26 | rs3844576 | <i>SOC1-TNP2</i> | 16 | 3.09E-06 |
| 27 | rs1793978 | <i>CKM-KLC3</i> | 19 | 2.70E-05 |

*The association of SNPs reported by the ImmunoChip study in Turkish patients.

†Some SNPs (six in total) were excluded since they were not polymorphic in Han Chinese (rs17753641, rs17810546, rs79891766, rs116379815, rs13190001 and rs1698386). The SNPs rs2647935, with $p < 5 \times 10^{-5}$, was selected as an alternative SNP in *IL12A*.

‡The SNPs identified by meta-analysis.

§Imputed SNPs that with more significant association than the lead SNPs in loci and that is not in high linkage disequilibrium with lead SNPs in Chinese Han population ($r^2 < 0.8$, Han Chinese Beijing).

SNP, single nucleotide polymorphism.

susceptibility SNPs that were identified by meta-analysis and imputation were also included in this study.

Some SNPs were excluded from this study for the following reasons. (1) The SNPs in the loci that have been reported previously by our team (*IL10*, *IL23R-IL12RB2*, *CCR1*, *ERAP1*, *KLRC4*, *IRF8*, *FOXP1*).¹⁴⁻¹⁸ (2) The SNPs that were not polymorphic in Han Chinese. (3) The SNPs that were in high linkage disequilibrium (LD) with the lead SNPs in Chinese Han ($r^2 > 0.8$, Han Chinese in Beijing, HCB) (table 1).

Genotyping

Genotyping of the selected 22 SNPs was performed in 478 BD cases and 662 controls drawn from a Chinese Han population in a first-stage study, and another independent cohort including 760 BD cases and 796 controls was examined in a second-stage confirmation study. Genotyping of 21 SNPs was performed using the MassARRAY platform (Sequenom, California, USA) and iPLEX Gold Assay. The SNPs rs1047781 was genotyped by TaqMan SNP Genotyping Assay (Applied Biosystems, Foster

City, California, USA) and the probe fluorescence signal was detected using the 7500 Real-Time PCR System (Applied Biosystems, USA). All SNPs tested had a high call rate ($\geq 95\%$ in all individual) and conformed to Hardy-Weinberg equilibrium (HWE) in the normal controls (p for HWE ≥ 0.05).

Statistical analysis

The χ^2 test was applied for the evaluation of the HWE. Genotype and allele frequencies were compared between patients with BD and normal controls by the χ^2 test or Fisher's exact test using SPSS V.17.0. The Bonferroni correction method was applied for the correction of p values for multiple comparisons. HWE was tested by the SHEsis website. Meta-analysis in multiple populations was performed using STATA software V.12.0. The p value of heterogeneity and I^2 were calculated to evaluate heterogeneity between populations. $P_{\text{het}} < 0.05$ and $I^2 > 0.5$ were considered to be significant. Statistical power was estimated from effect size in the original Turkish data sets from the 'Takeuchi' study,¹² allele frequency and sample size in the Chinese Han population. Statistical power analysis was performed using PS Power and Sample Size Calculations software (V.3.1.2; Department of Biostatistics, Vanderbilt University, Nashville, Tennessee, USA).

RESULTS

Clinical characteristics of patients with BDs

All 1238 patients with BD had uveitis, of which 26% patients had hypopyon. The most frequent type of uveitis was panuveitis (93.2%), followed by posterior uveitis (4.9%) and anterior uveitis (1.9%). Oral ulcers (94.4%) were the most frequent extraocular manifestation, followed by skin lesions (75.2%) and genital ulcers (55.4%). The distribution of age and gender and clinical features of the enrolled patients with BD and the healthy controls in this study are shown in table 2.

Association test of examined SNPs in the first phase

In the first-stage study, 22 SNPs were genotyped in 478 patients with BD and 662 normal controls. Significant higher frequencies of the *CEBPB-PTPN1*/rs913678 C allele ($p_c = 1.01 \times 10^{-2}$, OR=1.382), *RIPK2*/rs10094579 A allele ($p_c = 3.16 \times 10^{-2}$, OR=1.368) and *ADO-EGR2*/rs224127 A allele ($p_c = 3.36 \times 10^{-2}$, OR=1.318) were observed in patients

Table 2 Clinical features of patients with ocular Behcet's disease (BD) and controls enrolled in the study

| Clinical features | Number | Percentage (%) |
|------------------------|-----------|----------------|
| Patients with BD | 1238 | |
| Age (years), mean±SD | 33.9±9.1 | |
| Male | 1001 | 80.9 |
| Female | 237 | 19.1 |
| Uveitis | 1238 | 100 |
| Oral ulcer | 1169 | 94.4 |
| Genital ulcer | 686 | 55.4 |
| Arthritis | 223 | 18 |
| Skin lesions | 931 | 75.2 |
| Positive pathergy test | 47 | 3.8 |
| Controls | 1458 | |
| Age (years), mean±SD | 35.3±10.2 | |
| Male | 1191 | 81.7 |
| Female | 267 | 18.3 |

Table 3 Main effects of tested SNPs on BD risk

| Nearest gene(s) | SNP | Stage | Genotype/allele | Case | freq | Control | freq | P values | P _c values | OR (95% CI) | Statistical power |
|--------------------|------------|----------|-----------------|------|-------|---------|-------|----------|-----------------------|------------------------|-------------------|
| <i>CEBPB-PTPN1</i> | rs913678 | Stage1 | CC | 251 | 0.526 | 280 | 0.423 | 5.69E-04 | 3.75E-02 | 1.515 (1.196 to 1.920) | 0.998 |
| | | | CT | 186 | 0.390 | 303 | 0.458 | 2.26E-02 | NS | 0.757 (0.596 to 0.962) | |
| | | | TT | 40 | 0.084 | 79 | 0.119 | 0.054 | – | 0.675 (0.453 to 1.008) | |
| | | | C | 688 | 0.721 | 863 | 0.652 | 4.59E-04 | 1.01E-02 | 1.382 (1.153 to 1.656) | |
| | | Stage2 | CC | 405 | 0.534 | 379 | 0.476 | 2.35E-02 | NS | 1.259 (1.031 to 1.536) | |
| | | | CT | 297 | 0.391 | 337 | 0.423 | 0.198 | – | 0.876 (0.715 to 1.072) | |
| | | | TT | 57 | 0.075 | 80 | 0.101 | 0.077 | – | 0.727 (0.509 to 1.037) | |
| | | | C | 1107 | 0.729 | 1095 | 0.688 | 1.11E-02 | 4.43E-02 | 1.222 (1.047 to 1.428) | |
| | | Combined | CC | 656 | 0.531 | 659 | 0.452 | 4.60E-05 | 3.04E-03 | 1.371 (1.178 to 1.596) | |
| | | | CT | 483 | 0.391 | 640 | 0.439 | 1.15E-02 | NS | 0.820 (0.703 to 0.956) | |
| | | | TT | 97 | 0.078 | 159 | 0.109 | 7.01E-03 | NS | 0.696 (0.534 to 0.907) | |
| | | | C | 1795 | 0.726 | 1958 | 0.671 | 1.37E-05 | 3.01E-04 | 1.297 (1.154 to 1.459) | |
| <i>LACC1</i> | rs9316059 | Stage1 | TT | 17 | 0.036 | 56 | 0.085 | 7.87E-04 | 5.19E-02 | 0.397 (0.228 to 0.692) | 0.970 |
| | | | TA | 170 | 0.357 | 292 | 0.445 | 2.95E-03 | NS | 0.693 (0.543 to 0.883) | |
| | | | AA | 289 | 0.607 | 308 | 0.470 | 4.68E-06 | 3.09E-04 | 1.746 (1.374 to 2.219) | |
| | | | T | 204 | 0.214 | 404 | 0.308 | 6.96E-07 | 1.53E-05 | 0.613 (0.505 to 0.744) | |
| | | Stage2 | TT | 46 | 0.061 | 62 | 0.078 | 0.176 | – | 0.762 (0.513 to 1.131) | |
| | | | TA | 260 | 0.342 | 334 | 0.420 | 1.55E-03 | 1.86E-02 | 0.718 (0.584 to 0.882) | |
| | | | AA | 454 | 0.597 | 399 | 0.502 | 1.56E-04 | 1.87E-03 | 1.473 (1.205 to 1.808) | |
| | | | T | 352 | 0.232 | 458 | 0.288 | 3.35E-04 | 1.34E-03 | 0.745 (0.634 to 0.875) | |
| | | Combined | TT | 63 | 0.051 | 118 | 0.081 | 1.76E-03 | NS | 0.607 (0.442 to 0.832) | |
| | | | TA | 430 | 0.348 | 626 | 0.431 | 9.95E-06 | 6.56E-04 | 0.703 (0.601 to 0.822) | |
| | | | AA | 743 | 0.601 | 707 | 0.487 | 3.57E-09 | 2.36E-07 | 1.586 (1.360 to 1.849) | |
| | | | T | 556 | 0.225 | 862 | 0.297 | 2.25E-09 | 4.95E-08 | 0.687 (0.607 to 0.777) | |
| <i>RIPK2</i> | rs10094579 | Stage1 | AA | 29 | 0.061 | 30 | 0.045 | 0.246 | – | 1.336 (0.806 to 2.302) | 0.996 |
| | | | CA | 205 | 0.431 | 228 | 0.345 | 3.52E-03 | NS | 1.433 (1.125 to 1.826) | |
| | | | CC | 242 | 0.508 | 402 | 0.609 | 7.27E-04 | 4.80E-02 | 0.664 (0.523 to 0.842) | |
| | | | A | 263 | 0.276 | 288 | 0.218 | 1.44E-03 | 3.16E-02 | 1.368 (1.128 to 1.659) | |
| | | Stage2 | AA | 58 | 0.077 | 47 | 0.059 | 0.157 | – | 1.332 (0.895 to 1.984) | |
| | | | CA | 294 | 0.393 | 273 | 0.344 | 4.95E-02 | NS | 1.231 (1.000 to 1.514) | |
| | | | CC | 397 | 0.530 | 473 | 0.596 | 8.56E-03 | NS | 0.763 (0.624 to 0.934) | |
| | | | A | 410 | 0.274 | 367 | 0.231 | 6.84E-03 | 2.74E-02 | 1.252 (1.064 to 1.473) | |
| | | Combined | AA | 87 | 0.071 | 77 | 0.053 | 0.053 | – | 1.366 (0.995 to 1.875) | |
| | | | CA | 499 | 0.407 | 501 | 0.345 | 8.58E-04 | NS | 1.306 (1.116 to 0.528) | |
| | | | CC | 639 | 0.522 | 875 | 0.602 | 2.79E-05 | 1.84E-03 | 0.720 (0.618 to 0.840) | |
| | | | A | 673 | 0.275 | 655 | 0.225 | 3.15E-05 | 6.93E-04 | 1.302 (1.149 to 1474) | |
| <i>ADO-EGR2</i> | rs224127 | Stage1 | GG | 67 | 0.140 | 121 | 0.185 | 4.40E-02 | NS | 0.717 (0.518 to 0.992) | 0.985 |
| | | | GA | 217 | 0.454 | 324 | 0.496 | 0.161 | – | 0.844 (0.666 to 1.070) | |
| | | | AA | 194 | 0.406 | 208 | 0.319 | 2.44E-03 | NS | 1.461 (1.143 to 1.869) | |
| | | | A | 605 | 0.636 | 740 | 0.567 | 1.53E-03 | 3.36E-02 | 1.318 (1.111 to 1.564) | |
| | | Stage2 | GG | 90 | 0.118 | 135 | 0.170 | 3.73E-03 | 4.48E-02 | 0.655 (0.491 to 0.873) | |
| | | | GA | 366 | 0.482 | 378 | 0.477 | 0.847 | – | 1.020 (0.836 to 1.245) | |
| | | | AA | 304 | 0.400 | 280 | 0.353 | 5.64E-02 | – | 1.221 (0.994 to 1.500) | |
| | | | A | 974 | 0.641 | 938 | 0.591 | 4.70E-03 | 1.88E-02 | 1.232 (1.066 to 1.425) | |
| | | Combined | GG | 157 | 0.127 | 256 | 0.177 | 3.25E-04 | 2.14E-02 | 0.675 (0.544 to 0.837) | |
| | | | GA | 583 | 0.471 | 702 | 0.485 | 0.452 | – | 0.943 (0.810 to 1.098) | |
| | | | AA | 498 | 0.402 | 488 | 0.337 | 5.20E-04 | 3.43E-02 | 1.321 (1.129 to 1.546) | |
| | | | A | 1579 | 0.638 | 1678 | 0.580 | 1.71E-05 | 3.77E-04 | 1.274 (1.141 to 1.422) | |

Statistical power was estimated from effect size in the original Turkish data sets, allele frequency and sample size in the Chinese Han population.

BD, Behcet's disease; p_c value, the Bonferroni corrected p value; NS, not significant; SNP, single nucleotide polymorphism.

with BD. In addition, the frequency of the T allele for *LACC1*/rs9316059 was significantly lower in patients with BD (p_c=1.53×10⁻⁵; OR=0.613) (table 3). However, there was no association between the remaining SNPs and BD in this Chinese Han cohort (online supplementary table 1).

Association test of examined SNPs in the second phase and combined study

To further confirm the outcome of the first-stage study, we enrolled a separate set of 760 patients with BD and 796 healthy individuals for a second-stage test. We only tested the SNPs that

showed a significant association in the first phase. The frequency of the T allele for *LACC1*/rs9316059 in patients with BD was confirmed to be significantly lower ($p_c=1.34\times 10^{-3}$, OR=0.745). In addition, the result again demonstrated significantly higher frequencies of the *CEBPB-PTPN1*/rs913678 C allele ($p_c=4.43\times 10^{-2}$, OR=1.222), *RIPK2*/rs10094579 A allele ($p_c=2.74\times 10^{-2}$, OR=1.252) and *ADO-EGR2*/rs224127 A allele ($p_c=1.88\times 10^{-2}$, OR=1.232) in patients with BD (table 3).

Combination of the data from the first-stage and second-stage study showed that four SNPs (rs913678, rs9316059, rs10094579 and rs224127) were significantly associated with BD (rs913678 C allele: $p=1.37\times 10^{-5}$, $p_c=3.01\times 10^{-4}$, OR=1.297; rs9316059 T allele: $p=2.25\times 10^{-9}$, $p_c=4.95\times 10^{-8}$, OR=0.687; rs10094579 A allele: $p=3.15\times 10^{-5}$, $p_c=6.93\times 10^{-4}$, OR=1.302; rs224127 A allele: $p=1.71\times 10^{-5}$, $p_c=3.77\times 10^{-4}$, OR=1.274) (table 3).

Stratified analysis for rs913678, rs9316059, rs10094579 and rs224127

We also analysed whether rs913678, rs9316059, rs10094579 and rs224127 showed an association with the main clinical features of BD. The results did not show any significant association between the four tested SNPs and groups of patients with BD divided according to their clinical features (online supplementary table 2).

Meta-analysis

To further investigate the risk conferred by the SNPs (rs913678, rs9316059, rs10094579 and rs224127) associated with BD, we performed a meta-analysis of the genetic polymorphisms for which data were available from the Takeuchi association study and our study data sets. The results showed that the disease association of tested SNPs were reinforced after meta-analysis and all exceeded genome-wide significance (rs913678: $p=2.09\times 10^{-16}$; rs9316059: $p=2.96\times 10^{-16}$; rs10094579: $p=9.21\times 10^{-11}$; rs224127: $p=5.28\times 10^{-13}$) (table 4).

DISCUSSION

In the present study, we performed a replication study in a Han Chinese BD cohort for 22 candidate SNPs identified with an association p value $<5\times 10^{-5}$ with BD in a recent Immunochip study.¹² The results showed that four SNPs (rs913678 in

CEBPB-PTPN1, rs9316059 in *LACC1*, rs10094579 in *RIPK2*, rs224127 in *ADO-EGR2*) contribute to the genetic susceptibility to BD in a Chinese Han population.

LACC1/rs9316059, the most significantly associated SNP with BD in our study, displayed genome-wide significant association (table 3). The Immunochip study was performed in Turkish patients, and the findings concerning *LACC1* rs9316059 was also confirmed in a Japanese cohort.¹² In the same study, the association with SNP rs2121033 in *LACC1* was identified by meta-analysis in three populations (Turkish, Iranian and Japanese). These two SNPs are in strong LD with each other ($r^2=0.891$, in HCB). This study in combination with ours indicates that the protective *LACC1* locus is a commonly associated gene for BD in all the populations tested including Chinese Han, Turkish, Iranian and Japanese. In addition, we identified the susceptibility SNP rs10094579 in *RIPK2* in our cohort (table 3). To our knowledge, this is the first report showing that rs10094579 in *RIPK2* confers risk to BD. This SNP only showed a suggestive disease association with a Turkish BD cohort but was not confirmed both in an Iranian and Japanese BD cohort.¹² Moreover, the association we found for the other two SNPs (*CEBPB-PTPN1*/rs913678 and *ADO-EGR2*/rs224127) are in agreement with data in an Iranian population for *CEBPB-PTPN1*/rs913678 and a Japanese population for *ADO-EGR2*/rs224127 (table 3). In a meta-analysis of populations (table 4 and figure 1), we show that *ADO-EGR2*/rs224127, *CEBPB-PTPN1*/rs913678 and *PIP2K2*/rs10094579 all exceeded genome-wide significance. Based on these findings, we propose that *LACC1*, *CEBPB-PTPN1*, *RIPK2* and *ADO-EGR2* constitute BD susceptibility genes in Chinese Han, together with other established loci such as *IL10*, *IL23R-IL12RB2*, *CCR1*, *IRF8*, *KLRC4*, *STAT4*, *ERAP1*, *TNFAIP3*, *TNFSF4*, *UBAC2*, *IL-37*, *IL-18RAP*, *GAS6*, *PROS1*, *CD6*, *CD11c*, *ATG5*, *TRAF5*, *TRAF3IP2*, *JAK1*, *MIF*, *PDGFR*, *CD40*, *CIITA*, *NOD1*, *NOS3*, *REL* and *TLR2*.¹⁴⁻³⁴

Of note, we also did not find evidence supporting the disease association with two other reported loci (*IL1A-IL1B* and *FUT2*), which both showed a genome-wide association ($p<5\times 10^{-8}$) in the original 'Immunochip' report.¹² *IL1A-IL1B*/rs3783550 that was identified in Turkish patients could also not be confirmed in Iranian as well as Japanese patients with BD. We could also not confirm the association with the *FUT2*/rs1047781 (T) allele,

Table 4 Meta-analysis of multiple populations for the markers replicated in the Han Chinese cohorts

| Marker (loci) | Risk allele | Population | OR | 95% CI | P values | I ² | P _{het} values |
|---------------------------------|-------------|---------------|------|--------------|----------|----------------|-------------------------|
| rs913678 (<i>CEBPB-PTPN1</i>) | C | Turkish | 1.33 | 1.21 to 1.46 | 1.10E-09 | 0 | 0.92 |
| | | Iranian | 1.29 | 1.13 to 1.48 | 1.59E-04 | | |
| | | Han Chinese | 1.30 | 1.15 to 1.46 | 1.37E-05 | | |
| | | Meta-analysis | 1.31 | 1.23 to 1.40 | 2.09E-16 | | |
| rs9316059 (<i>LACC1</i>) | T | Turkish | 0.79 | 0.71 to 0.88 | 1.16E-05 | 0.45 | 0.16 |
| | | Japanese | 0.67 | 0.56 to 0.82 | 5.41E-05 | | |
| | | Han Chinese | 0.69 | 0.61 to 0.78 | 2.25E-09 | | |
| | | Meta-analysis | 0.73 | 0.68 to 0.79 | 2.96E-16 | | |
| rs10094579 (<i>RIPK2</i>) | A | Turkish | 1.34 | 1.19 to 1.50 | 6.03E-07 | 0 | 0.73 |
| | | Han Chinese | 1.30 | 1.15 to 1.47 | 3.15E-05 | | |
| | | Meta-analysis | 1.32 | 1.21 to 1.44 | 9.21E-11 | | |
| rs224127 (<i>ADO-EGR2</i>) | A | Turkish | 1.26 | 1.15 to 1.39 | 1.56E-06 | 0 | 0.94 |
| | | Japanese | 1.30 | 1.11 to 1.51 | 1.10E-03 | | |
| | | Han Chinese | 1.27 | 1.14 to 1.42 | 1.71E-05 | | |
| | | Meta-analysis | 1.27 | 1.19 to 1.36 | 5.28E-13 | | |

Meta-analysis was performed for populations in which association for the variant exceeded the replication threshold.

I², inconsistency index; p_{het}, p for heterogeneity.

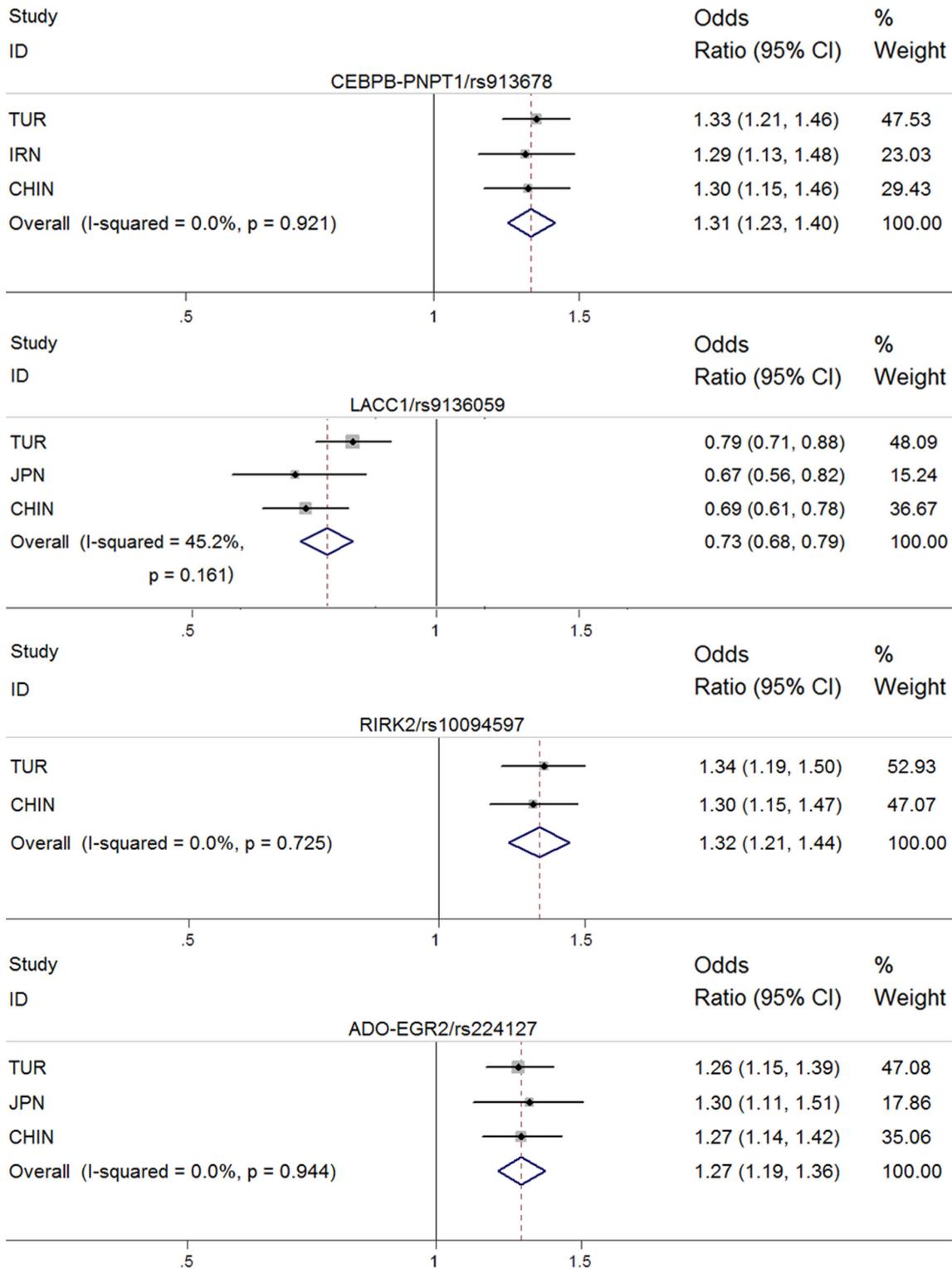


Figure 1 Forest plots for four SNPs associated with BD in the Chinese Han population compared with other populations. For the four SNPs, the meta-analysis refer to the C, T, A and A alleles, respectively. The broken vertical line shows the no effect point (OR 1). BD, Behcet’s disease; CHIN, HanChinese; JPN, Japanese; SNP, single nucleotide polymorphism; TUR, Turkish.

which is an ancestry-specific *FUT2* non-secretor mutation (in Japanese and Han Chinese) with a significant association with BD in Japanese.¹² The fact that the two SNPs mentioned above show a lack of association in Chinese Han is probably due to the different genetic background between Chinese Han and Japanese and Turkish populations, since our sample size was large enough to find a possible existing association (Power>0.8) (online

supplementary table 1). Populations between different continents show between 16% and 19% genetic differences. Even within a continent, populations may differ genetically, whereby Japanese and Chinese, differ by 6.78%.³⁵ Similarly, we did not confirm an association with the other two SNPs (rs1509966 and rs7075773) in *ADO-EGR2* and BD susceptibility. Although they had a relatively high statistic power of 0.641 and 0.738,

respectively, we could not exclude a false-negative disease association of rs1509966 and rs7075773 in our study. In addition, the difference of the associations for three SNPs in *ADO-EGR2* among the nationalities may be partly explained by the population genetic heterogeneity. Likewise, *RIPK2*/rs2230801, another SNP identified in Turkish patients and confirmed in Japanese patients, did not show a significant association with BD in our study. Given that rs2230801 is a rare variant in China (minor allele frequency < 0.05), the statistical power to confirm this finding was low (0.23) (online supplementary table 1).

A recent study reported that the *LACC1*/rs3764147 (p.Ile254Val) is in high LD with rs9316059 ($r^2=0.892$, HCB) and leads to impaired protein function.³⁶ Furthermore, *Lacc1*^{-/-} mice produce decreased IL-1 β in response to lipopolysaccharide treatment, consistent with a role for *IL-1 β* in BD pathogenesis.³⁶ The minor allele of the other three SNPs increase the risk for BD. The *CEBPB-PTPN1*/rs913678 C allele is associated with decreased gene expression, and *Cebpb*^{-/-} mice show increased susceptibility to pathogens.^{37,38} The *RIPK2* kinase transduces signalling downstream of the intracellular peptidoglycan sensors NOD1 and NOD2 to promote a productive inflammatory response.³⁹ However, excessive NOD2 signalling has been associated with numerous diseases, including inflammatory bowel disease (IBD), sarcoidosis and inflammatory arthritis.⁴⁰⁻⁴² Interestingly, *ADO-EGR2* has also been identified as a risk for Vogt-Koyanagi-Harada (VKH) syndrome by a previous genome-wide association study of our team.⁴³ *ADO* and *EGR2* were all expressed in the iris, whereas *EGR2* was also expressed in ciliary body and choroid.⁴³ VKH syndrome and BD are two of the most common types of uveitis in Chinese Han, and the fact that they share common susceptibility loci suggests that *ADO-EGR2* may be a common genetic locus for uveitis, which may provide a theoretical basis for prevention and treatment of other type of uveitis.

The four novel BD susceptibility loci that we could confirm in Chinese patients have also been reported to be associated with other immune disorders. *ADO-EGR2*, *LACC1* and *CEBPB-PTPN1* are shared by BD and IBD.⁴¹⁻⁴⁴ *RIPK2*, *ADO-EGR2* and *LACC1* have been shown to be associated with leprosy.⁴⁷⁻⁵⁰ These observations suggest that these diseases, whether being autoinflammatory (BD and IBD) or infectious (leprosy), may share molecular pathways although their exact role (protective or susceptibility) may differ markedly. The C allele of rs913678 in *CEBPB-PTPN1* confers risk of BD but was protective for IBD and ulcerative colitis (UC).⁴¹⁻⁴⁶ The minor allele of *LACC1*/rs9316059 confers protection for BD but is in high LD with a common coding variant, rs3764147 ($r^2=0.892$, HCB), which increases risk for IBD, Crohn's disease (CD) and leprosy.⁴¹⁻⁴⁹ A similar discrepancy is also seen in *RIPK2*, where the minor allele of rs10094579 conferred risk of BD but is in high LD with rs7015630 ($r^2=0.976$, HCB) that was protective for CD and leprosy.⁴¹⁻⁴⁹ These discordant observations suggest that these genes which are involved in various signalling pathways may play opposite roles in BD as compared with IBD and leprosy. Further functional investigations may help to elucidate the molecular mechanisms underlying BD development and increase our understanding on the impact of these loci in the pathogenesis of autoinflammatory and infectious diseases.

Our study has several limitations. We only replicated the lead SNPs from the loci identified by a previous ImmunoChip study¹² and we cannot exclude that other suggestive SNPs may show an association with BD in Chinese Han. In addition, there were four genes that did not show informative results. These included *LONRF2*, *RBM6* and *C5orf56* since the only SNP included was

not polymorphic, and *IL12A*, where the two SNPs included, had one that was not polymorphic and data analysis of the other SNP *IL12A*/rs2647935 showed a statistical power that was too low to obtain a meaningful conclusion. Further, fine mapping for these gene regions is needed in Chinese Han to definitely show whether this is not a false-negative association. It should also be noted that all the patients with BD in the present study suffered from uveitis (100%), whereas the Turkish, Iranian and Japanese patients from the 'ImmunoChip' association study showed a lower uveitis incidence (39.4%, 56.3%, 86.9%, respectively),¹² which indicates that there may be a selection bias towards ocular BD in our study. One should also be aware of the fact that most of our patients with BD are male (80.9%), which is in agreement with the previous reports from countries along the ancient Silk Road,⁵¹⁻⁵³ whereas studies from Europe or the USA often show an almost equal gender distribution.⁵⁴⁻⁵⁶

In conclusion, our study not only confirms the association of *LACC1*/rs9316059, *CEBPB-PTPN1*/rs913678 and *ADO-EGR2*/rs224127 with BD but also identifies a novel *RIPK2*/rs10094579 polymorphism that affects BD susceptibility in Chinese Han. Our findings are an addition to the growing body of data from different ethnic populations, thereby gradually revealing the genetic risk landscape of BD. Further investigations on how these gene polymorphisms exactly affect BD are needed and may provide future targets for its treatment.

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Competing interests None declared.

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