Relationships of HLA-B51 or B5 genotype with Behçet’s disease clinical characteristics: systematic review and meta-analyses of observational studies

Carla Maldini¹, Michael P. LaValley², Morgane Cheminant¹, Mathilde de Menthon¹ and Alfred Mahr¹

Abstract

Objective. To investigate comprehensively the relationships between Behçet’s disease (BD) clinical features and HLA-B51 or HLA-B5 (HLA-B51/B5) status using meta-analyses.

Methods. Relevant publications were identified by a systematic literature search. Eligible studies had to provide frequencies for one or more BD characteristics according to HLA-B51/B5 status. Pooled relative risks (RRs) were calculated by random-effects meta-analysis for those BD characteristics for which five or more relevant studies were identified. Between-study variability was assessed with $I^2$ and $Q$-statistics, and modelled using meta-regression.

Results. Among the 859 publications evaluated, 72 (representing 74 study populations) met eligibility criteria. Pooled RRs (95% CIs) of the association of HLA-B51/B5 with the 14 analysed clinical characteristics were male sex 1.14 (1.05, 1.23); eye involvement 1.13 (1.06, 1.21); genital ulcers 1.07 (1.01, 1.14); skin involvement 1.10 (1.03, 1.16); erythema nodosum 1.11 (0.96, 1.29); pseudofolliculitis 1.07 (0.93, 1.23); positive pathergy test 1.05 (0.94, 1.17); joint involvement 0.94 (0.86, 1.04); neurological involvement 0.95 (0.71, 1.27); gastrointestinal involvement 0.70 (0.52, 0.94); thrombophlebitis 1.17 (0.77, 1.76); vascular involvement 1.00 (0.68, 1.47); chest involvement 1.55 (0.75, 3.20) and orchiepididymitis 1.13 (0.59, 2.15). For most of the analysed outcomes, between-study heterogeneity was low or absent and most of the meta-regression models were statistically non-significant.

Conclusion. The results of these meta-analyses showed that, in BD, HLA-B51/B5 carriage predominates in males and is associated with moderately higher prevalences of genital ulcers, ocular and skin manifestations, and a decreased prevalence of gastrointestinal involvement.

Key words: Behçet’s disease, human leucocyte antigens, genetics, meta-analysis.

Introduction

Behçet’s disease (BD) is a rare chronic, inflammatory, multisystem disorder predominantly affecting populations of Asian, Middle Eastern and Mediterranean ancestry. With the exception of oral aphthosis, BD is characterized by considerable phenotypic variation, comprising a myriad of manifestations, e.g. recurrent genital ulcers and skin, joint, eye, vascular and/or CNS involvement. Over the last 30 years, a substantial body of knowledge has accumulated supporting a strong genetic underpinning in BD of the MHC-related allele HLA-B5, which was later more specifically linked to its predominant suballele HLA-B51 [1, 2]. HLA-B51 or HLA-B5 (henceforth denoted HLA-B51/B5) is carried by one- to two-thirds of patients and increases the risk of BD development by a factor of about 6 [3].

The protean nature of clinical BD manifestations raised the question of whether HLA-B51/B5 also has a modulatory effect on disease expression. Study results suggested that HLA-B51/B5-positive and negative BD
patients differed in that the former more frequently de-
veloped CNS [4] or eye involvement [2, 5] and the latter
more commonly thrombophlebitis [6]. In addition, it
was suggested that patients harbouring the HLA-B51/B5
allele have more unfavourable BD courses, characterized
by poorer outcomes of ocular [4, 7, 8] or neurological
involvement [4]. However, these observations have not
been reported consistently [9, 10] and the discrepancies
may have been exacerbated by studies with small sample
sizes. In light of the potential implications of this mat-
er in clinical practice and for the understanding of BD
pathogenesis, a meta-analysis of observational studies
appeared suitable to elucidate the relationships between
the HLA-B51/B5 genetic background and clinical BD
phenotype. We report the results of a systematic litera-
ture review and meta-analyses of observational case
studies in an attempt to clarify potential relationships
between HLA-B51/B5 and BD-related phenotypic
manifestations.

Methods

Data sources and searches

We searched the literature for BD case studies using the
PubMed MEDLINE and Embase databases to identify
publications relevant to the purpose of this study, without
any language restriction. The search period was January
1973 through September 2008. Various combinations of
the following medical subject headings and keywords
were used in searching: Behçet’s disease, Behçet’s syn-
drome, HLA-B5, HLA-B51, and HLA-B51. Reference lists from
the retrieved publications and references identified in a
previous systematic literature review on the HLA-B51/B5
association with BD [3] were also reviewed and additional
Google Internet searches were performed.

Study selection and data extraction

Eligible publications were those providing information on
the distribution of one or more phenotypic BD character-
istics, defined as demographic characteristics (age, sex)
and generic or specific organ or system manifestations,
according to HLA-B51/B5 status of BD cases. From each
eligible publication, two readers (C.M., M.C.) independ-
ently gathered data using a structured data collection
form. In addition to the distributions of cases with and
without the relevant clinical variable(s) according to
HLA-B51/B5 status, the form included information on
author and study area, publication year, type and lan-
guage, BD classification criteria used, screened allele
and genotyping technique. Between-reader inconsistenc-
ies in the collected information were resolved by discus-
sion and re-analysis of the original data and, when
necessary, third-party adjudication (A.M.) until a consen-
sus was reached. Any publication reporting on less than
10 BD patients was excluded a priori.

When several reports were published by the same
research centres, we assessed potential overlap of patient
data to avoid inclusion of publications analysing the same
or overlapping subjects. Unless this information was
explicitly given in multiple publications from the same in-
stitution, the authors were contacted personally or by
e-mail for clarification. For studies with overlapping sub-
jects reporting on the same genotype-phenotype out-
comes, we used the data from the publication reporting
on the highest number of individuals. Selected geno-
type–phenotype data from smaller overlapping publica-
tions were also taken into consideration when they had
not been given in the larger publication.

Subsequently, all clinical variables for which relevant
information was identified were categorized into homoge-
neous groups. The findings reported on the features
orchitis and epididymitis were combined under the term
orchiepididymitis.

Data synthesis and analysis

A meta-analysis was generated for each clinical variable
for which five or more studies had been identified with
relevant information of its distribution as a function of
HLA-B51/B5 status. For each meta-analysis, we calcu-
lated the relative risk (RR) and the 95% CIs for individual
studies and estimated the pooled RR (95% CI) using fixed-
and random-effects meta-analysis models [11]. Although
both models yielded similar estimates in most instances,
only random-effects results are presented herein because
between-study heterogeneity was identified in some
meta-analyses. Heterogeneity across studies was evalu-
ated with the DerSimonian-Laird $\chi^2$-based $Q$-statistic [12].
We also computed the $I^2$-statistic, which is the percent-
age of total variation of RR estimates attributable to
between-study heterogeneity, rather than sampling error
[13, 14]. To assess the possibility of publication bias, we
used the Egger test [15], the Begg test (adjusted
rank-correlation test) [16] and contour-enhanced funnel
plots [17]; the latter were assessed visually for symmetry
and location of study values relative to contours deter-
mined by significance levels of 0.1, 0.05 and 0.01.

The robustness of the observed results was evaluated
by a pre-defined sensitivity analysis in which only studies
with 50 or more BD subjects were included in the
meta-analyses. Following the same algorithm as that
applied to the primary analyses, meta-analyses were gen-
erated exclusively for clinical variables with at least five
informative studies. To further explore the impact of spe-
cific study-level characteristics on the RR for BD charac-
teristics by HLA-B51/B5 carriage, we undertook univariate
random-effects meta-regression, using male sex, genital
ulcers, eye and skin involvement as dependent variables
and geographic area (stratified by Asia, Middle East/North
Africa and Europe), HLA-B51 vs HLA-B5 genotype, sero-
logical vs molecular HLA testing, publication language
(English vs other), publication type (peer-reviewed article
vs publication in books/conference proceedings) and year
of publication as explanatory variables.

Within each of the meta-analysis data sets, we also
calculated the pooled prevalence, expressed as percent-
ages (95% CI), of the corresponding clinical variable and
the pooled prevalences of HLA-B51/B5. In addition to the
calculations based on the entire data sets, prevalences of
clinical variables were computed within the HLA-B51/B5-positive and negative subgroups. These calculations also used random-effects meta-analysis techniques, as previously described [18].

All statistical analyses were conducted with SAS (version 9.1; Cary, NC, USA). Statistical tests were two-tailed and statistical significance was defined as $P < 0.05$, except for the tests for publication bias, whose significance level was set at a conservative value of $P < 0.10$.

Results

Search results and description of studies

Fig. 1 summarizes the study selection process. Among 859 titles, abstracts or full reports identified and reviewed, 769 did not satisfy the pre-stated inclusion criteria. Among the 90 potentially relevant articles, 13 were excluded [19-31] because they contained fully overlapping information with one or several other publications. Five other studies were excluded because they informed on outcome variables, i.e. oral ulcers [32-34], arterial involvement [35] and/or age [34, 36], which did not qualify for performing a meta-analysis (see below).

Finally, 72 articles were retained that contributed data to one or more clinical BD-characteristic genotype relationships [2, 5-10, 37-101]. From 14 publications [10, 37, 39, 41, 52, 69, 71, 84, 86, 96-98, 100, 101], only selected information on all available clinical BD phenotype-genotype associations was used because these publications had overlapping patient data with other publications. Two of the 72 publications referred to paediatric series [67, 93]. For one study, whose reported BD cases included several related subjects, only the data on unrelated individuals were used [59]. Three reports included two geographical populations [47, 86, 97], which were considered separately; for one of them [97], data on one geographical population were not used because of an overlap with another publication. Thus the 72 publications retained concerned 74 patient populations.

Detailed characteristics of the 74 study populations used for the meta-analyses are shown in Table 1. Thirty-five study populations assessed the genotype-phenotype associations for HLA-B5 and 34 study populations for HLA-B51. In addition, four study populations provided information on both HLA-B5 and HLA-B51 [39, 44, 46, 57]; we used HLA-B5 data from three publications [44, 46, 57] and HLA-B51 data from the remaining study [39], because this approach maximized the numbers of informative cases. The results of another study, in which the HLA-B5101 suballele was genotyped, were assigned to the subcategory of studies with genotyped HLA-B51 [68]. Classification criteria used were the International Study Group (ISG) criteria [102] (27 study populations), 1974/1987 revised Japanese BD research committee (JBDRC) criteria [103, 104] (21 study populations), other criteria [75, 105-110] (18 study populations), or were study-specific or unstated (8 study populations). Most of the analysed publications were full-length reports in peer-reviewed journals (61 study populations) and were written in English (59 study populations). Geographical

Fig. 1 Flow diagram of the study selection process.
<table>
<thead>
<tr>
<th>Study</th>
<th>Reference type</th>
<th>Country</th>
<th>Area</th>
<th>Sample size</th>
<th>Criteria</th>
<th>Allele tested</th>
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<td>ISG</td>
<td>B51</td>
<td>Sex</td>
</tr>
<tr>
<td>Ning et al. [81]</td>
<td>PRJ</td>
<td>China</td>
<td>Asia</td>
<td>27</td>
<td>Not reported</td>
<td>B51</td>
<td>Sex</td>
</tr>
<tr>
<td>Nishiyama et al. [82]</td>
<td>PRJ</td>
<td>Japan</td>
<td>Asia</td>
<td>68</td>
<td>JBDRC</td>
<td>B51</td>
<td>GI, G-Ulc, joint, neuro, orch, PT, skin, vasc</td>
</tr>
<tr>
<td>Nishiyama et al. [83]</td>
<td>PRJ</td>
<td>Japan</td>
<td>Asia</td>
<td>2960</td>
<td>JBDRC</td>
<td>B51</td>
<td>Eye, sex</td>
</tr>
<tr>
<td>Ofno et al. [2]</td>
<td>PRJ</td>
<td>Japan</td>
<td>Asia</td>
<td>184</td>
<td>JBDRC</td>
<td>B5</td>
<td>Eye</td>
</tr>
<tr>
<td>Okinami et al. [85]</td>
<td>PRJ</td>
<td>Japan</td>
<td>Asia</td>
<td>45</td>
<td>JBDRC</td>
<td>B5</td>
<td>Eye</td>
</tr>
<tr>
<td>Okuyama et al. [86]</td>
<td>CP</td>
<td>Japan</td>
<td>Asia</td>
<td>19</td>
<td>JBDRC</td>
<td>B5</td>
<td>PT</td>
</tr>
<tr>
<td>Okuyama et al. [86]</td>
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<td>Italy</td>
<td>Europe</td>
<td>10</td>
<td>JBDRC</td>
<td>B5</td>
<td>EN, eye, GI, G-Ulc, joint, PT, sex</td>
</tr>
<tr>
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<td>Italy</td>
<td>Europe</td>
<td>112</td>
<td>ISG</td>
<td>B51</td>
<td>Sex</td>
</tr>
<tr>
<td>Pivetti Pezzi et al. [88]</td>
<td>PRJ</td>
<td>Italy</td>
<td>Europe</td>
<td>51</td>
<td>JBDRC</td>
<td>B5</td>
<td>Sex</td>
</tr>
<tr>
<td>Sekido et al. [89]</td>
<td>PRJ</td>
<td>Japan</td>
<td>Asia</td>
<td>32</td>
<td>JBDRC</td>
<td>B5</td>
<td>Eye</td>
</tr>
<tr>
<td>Si et al. [90]</td>
<td>PRJ</td>
<td>China</td>
<td>Asia</td>
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<td>Not reported</td>
<td>B5</td>
<td>Eye</td>
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<tr>
<td>Soylu et al. [8]</td>
<td>PRJ</td>
<td>Turkey</td>
<td>East</td>
<td>65</td>
<td>Not reported</td>
<td>B5</td>
<td>Eye</td>
</tr>
<tr>
<td>Takano et al. [91]</td>
<td>PRJ</td>
<td>Japan</td>
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<td>54</td>
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<td>B5</td>
<td>Sex</td>
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<tr>
<td>Terayama et al. [92]</td>
<td>PRJ</td>
<td>Japan</td>
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<td>B51</td>
<td>Eye, sex</td>
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<tr>
<td>Uziel et al. [93]</td>
<td>PRJ</td>
<td>Israel</td>
<td>East</td>
<td>26</td>
<td>Study-specific</td>
<td>B5</td>
<td>Chest, EN, eye, GI, G-Ulc, joint, neuro, PT, sex, skin, TPitis</td>
</tr>
<tr>
<td>Verity et al. [5]</td>
<td>PRJ</td>
<td>Jordan/Palestine</td>
<td>East</td>
<td>101</td>
<td>ISG</td>
<td>B51</td>
<td>Eye, sex</td>
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<tr>
<td>Wang et al. [94]</td>
<td>PRJ</td>
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<td>B51</td>
<td>Eye</td>
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<td>Wechsler et al. [95]</td>
<td>PRJ</td>
<td>France</td>
<td>Europe</td>
<td>50</td>
<td>JBDRC</td>
<td>B5</td>
<td>Chest, eye, GI, G-Ulc, joint, neuro, sex, skin, TPitis</td>
</tr>
<tr>
<td>Yazici et al. [96]</td>
<td>PRJ</td>
<td>Turkey</td>
<td>East</td>
<td>19</td>
<td>O’Duffy</td>
<td>B5</td>
<td>Neuro</td>
</tr>
<tr>
<td>Yazici et al. [97]</td>
<td>PRJ</td>
<td>UK</td>
<td>Europe</td>
<td>14</td>
<td>O’Duffy</td>
<td>B51</td>
<td>G-Ulc, joint, skin, TPitis</td>
</tr>
<tr>
<td>Yazici et al. [98]</td>
<td>PRJ</td>
<td>Turkey</td>
<td>East</td>
<td>49</td>
<td>O’Duffy</td>
<td>B5</td>
<td>Chest, G-Ulc, PT, skin</td>
</tr>
<tr>
<td>Yurdakul et al. [99]</td>
<td>PRJ</td>
<td>Turkey</td>
<td>East</td>
<td>19</td>
<td>Study-specific</td>
<td>B5</td>
<td>EN, G-Ulc, joint, PT, sex</td>
</tr>
<tr>
<td>Zouboulis et al. [100]</td>
<td>CP</td>
<td>Germany</td>
<td>Europe</td>
<td>40</td>
<td>ISG</td>
<td>B5</td>
<td>Chest, EN, GI, G-Ulc, joint, neuro, PT</td>
</tr>
<tr>
<td>Zouboulis et al. [101]</td>
<td>PRJ</td>
<td>Germany</td>
<td>Europe</td>
<td>64</td>
<td>Davatchi</td>
<td>B5</td>
<td>Sex, skin, TPitis, vasc</td>
</tr>
</tbody>
</table>

References for classification criteria: Curth [108], Davatchi et al. [109], ISG [103], JBDRC [103, 104], Mason and Barnes [105] and O’Duffy [110]. CP: conference proceedings; EN: erythema nodosum; GI: gastrointestinal; G-Ulc: genital ulcers; M. East: Middle East; neuro: neurological; N. Africa: North Africa; orch: orchepididymitis; PF: pseudofolliculitis; PRJ: peer-reviewed journal; PT: pathergy test; TPitis: thrombophlebitis; vasc: vascular.
distribution of the 74 study populations was Middle Eastern/North African countries 26, Asian nations 20 and European countries 28.

**Description of selected clinical characteristics**

Relevant information on the association with HLA-B51/B5 could be extracted from five or more study populations for the following 14 phenotypic characteristics: male sex (39 populations), genital ulcers (47 populations), skin involvement (24 populations), erythema nodosum (14 populations), pseudofolliculitis (6 populations), pathergy test (17 populations), joint involvement (29 populations), gastrointestinal involvement (29 populations), orchiepididymitis (5 populations), thrombophlebitis (18 populations), vascular involvement (12 populations) and chest involvement (6 populations). For each of these phenotypic characteristics, a list of the original parameter designations applied in the individual publications can be provided upon request.

In contrast, for the phenotypic variables—arterial disease, heart involvement, kidney involvement, myositis and audiovestibular involvement—five or fewer informative study populations were identified; hence these outcomes were not used for meta-analyses. For the outcome age (at disease onset, diagnosis or time of study), no meta-analysis was undertaken, due to insufficient data reported on variance, which precluded quantitative analyses. Also, no meta-analysis was generated for the variable oral ulcers because this manifestation is virtually present in all BD patients and also a mandatory ISG criterion [102]. We therefore thought it unlikely that HLA-B51/B5 influences the occurrence of oral ulcers, thereby rendering meta-analysis inappropriate.

**Relationships between HLA-B51/B5 status and clinical characteristics**

The number of subjects included in each of the 14 clinical phenotype-genotype relationship meta-analyses ranged from 143 to 5790. Table 2 provides the pooled RR for each given manifestation among HLA-B51/B5-positive BD subjects compared with those not carrying this allele. The forest plots of all the study-specific RR estimates and the summary-effect estimates for the outcomes eye involvement and male sex are shown in Fig. 2.

These meta-analyses indicated that HLA-B51/B5 carriage predominates in men (RR 1.14, 95% CI 1.05, 1.23, \( P = 0.001 \)), and increases the risk of genital ulcers (RR 1.07, 95% CI 1.01, 1.14, \( P = 0.03 \)), eye involvement (RR 1.13, 95% CI 1.06, 1.21, \( P < 0.0005 \)) and skin involvement (RR 1.10, 95% CI 1.03, 1.16, \( P = 0.003 \)), and decreases risk of gastrointestinal involvement (RR 0.70, 95% CI 0.52, 0.94, \( P = 0.02 \)). No statistically significant effects of HLA-B51/B5 on the other analysed phenotypic features were observed.

\( I^2 \)-statistics indicated moderate heterogeneity in the analyses for thrombophlebitis (\( I^2 = 56\% \)) and low

### Table 2

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Number of study populations</th>
<th>Number of subjects</th>
<th>Pooled prevalence (95% CI)</th>
<th>Effect size (RR, 95% CI)</th>
<th>Publication bias</th>
<th>Heterogeneity statistics</th>
<th>Publication bias</th>
<th>Heterogeneity statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye involvement</td>
<td>47</td>
<td>5790</td>
<td>63.5 (57.2, 69.3)</td>
<td>1.13 (1.06, 1.21)</td>
<td>&lt;0.0005</td>
<td>0.004</td>
<td>38.9</td>
<td>0.20</td>
</tr>
<tr>
<td>Male sex</td>
<td>39</td>
<td>2720</td>
<td>58.9 (49.4, 68.3)</td>
<td>1.14 (1.05, 1.23)</td>
<td>0.001</td>
<td>0.01</td>
<td>15.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Genital ulcers</td>
<td>30</td>
<td>1384</td>
<td>54.4 (47.7, 61.6)</td>
<td>1.07 (1.01, 1.14)</td>
<td>0.005</td>
<td>0.001</td>
<td>20.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>24</td>
<td>1352</td>
<td>76.8 (69.2, 84.5)</td>
<td>1.0 (1.03, 1.04)</td>
<td>0.003</td>
<td>0.001</td>
<td>6.7</td>
<td>0.77</td>
</tr>
<tr>
<td>Erythema nodosum</td>
<td>14</td>
<td>849</td>
<td>47.5 (38.6, 56.5)</td>
<td>1.11 (0.71, 1.77)</td>
<td>0.036</td>
<td>0.001</td>
<td>6.7</td>
<td>0.77</td>
</tr>
<tr>
<td>Pseudofolliculitis</td>
<td>6</td>
<td>458</td>
<td>68.7 (41.5, 84.5)</td>
<td>1.13 (0.93, 1.23)</td>
<td>0.036</td>
<td>0.001</td>
<td>56.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Pathergy test</td>
<td>17</td>
<td>913</td>
<td>54.1 (42.9, 62.3)</td>
<td>1.05 (0.94, 1.17)</td>
<td>0.036</td>
<td>0.001</td>
<td>56.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Thrombophlebitis</td>
<td>18</td>
<td>1322</td>
<td>19.9 (15.8, 25.5)</td>
<td>1.07 (0.77, 1.77)</td>
<td>0.036</td>
<td>0.001</td>
<td>56.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Vascular involve-ment</td>
<td>12</td>
<td>606</td>
<td>23.8 (16.6, 32.9)</td>
<td>1.07 (0.77, 1.47)</td>
<td>0.036</td>
<td>0.001</td>
<td>56.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Gastrointestinal involvement</td>
<td>14</td>
<td>849</td>
<td>23.8 (16.6, 32.9)</td>
<td>1.07 (0.77, 1.47)</td>
<td>0.036</td>
<td>0.001</td>
<td>56.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Chest involvement</td>
<td>6</td>
<td>252</td>
<td>9.2 (5.1, 16.2)</td>
<td>1.55 (0.75, 3.20)</td>
<td>0.036</td>
<td>0.001</td>
<td>56.4</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\( \text{Variables are ordered by decreasing number of individual study populations and subjects used for each of the 14 meta-analyses.} \)
heterogeneity for eye involvement ($I^2 = 39\%$) and erythema nodosum ($I^2 = 48\%$); $Q$-statistics were significant for eye involvement ($P = 0.004$), thrombophlebitis ($P = 0.001$) and erythema nodosum ($P = 0.02$). For all other variables, between-study heterogeneity assessed by $I^2$ was low or absent and the $Q$-statistic results were not significant.

The search for publication bias according to Begg and Egger tests (Table 2) and the patterns of the contour-enhanced funnel plots (not shown) suggested that publication bias was present in the meta-analyses of eye involvement, male sex and erythema nodosum, which could indicate that the pooled RR for these characteristics is somewhat inflated.

Sensitivity analyses that removed all studies that included 50 or fewer subjects were performed for 10 variables with five or more informative studies. These analyses yielded very similar RR point estimates to those obtained using the whole data sets (see supplementary Table S1, available at Rheumatology Online). In addition, after reanalysis of the association with eye involvement or male sex after removal of one very large study reporting on both variables [83], the pooled RR point estimates for these two variables were unchanged (data not shown).

The results of meta-regression analyses suggested that some of the variation of study results for associations of eye involvement or male sex with HLA-B51/B5 could be linked to study-level characteristics (Table 3). In particular, the RR values for eye manifestations and HLA-B51/B5 were higher when classification systems other than the ISG or JBDRC criteria were used when studies were conducted in Europe or when they were not published in English. The RR for association of male sex and HLA-B51/B5 was lower in studies conducted in the Middle East or North Africa (data not shown). None of the other BD features and study characteristics had significant associations in meta-regression analyses.

Frequency estimates of phenotypic characteristics and HLA-B51/B5

Based on the 14 meta-analysis data sets, we calculated pooled frequencies of the phenotypic BD characteristics and the pooled frequencies of HLA-B51/B5 carriage.
The results of these analyses are shown in Table 2. Fig. 3 shows the pooled frequencies of the phenotypic characteristics stratified by HLA-B51/B5 status. The estimated proportions of HLA-B51/B5-positive cases in these 14 population pools ranged from 49.8% (95% CI 30.9%, 68.7%) (for the pseudofolliculitis meta-analysis) to 63.8% (95% CI 45.7%, 78.7%) (for the chest involvement meta-analysis).

### Table 3: Meta-regression analyses assessing the effect of selected covariates on the pooled effect size of HLA-B51/B5 carriage on the risk for BD-related characteristics

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Eye involvement</th>
<th>Male sex</th>
<th>Genital ulcers</th>
<th>Skin involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of study populations</td>
<td>(P_{\text{cov}})</td>
<td>Number of study populations</td>
<td>(P_{\text{cov}})</td>
</tr>
<tr>
<td>Peer-reviewed journal (yes/no)</td>
<td>39/8</td>
<td>0.40</td>
<td>33/6</td>
<td>0.49</td>
</tr>
<tr>
<td>Classification criteria (three categories)(a)</td>
<td>15/15/17</td>
<td>0.03</td>
<td>15/10/14</td>
<td>0.81</td>
</tr>
<tr>
<td>Geographic location (three categories)(b)</td>
<td>13/14/20</td>
<td>0.01</td>
<td>9/10/20</td>
<td>0.04</td>
</tr>
<tr>
<td>Allele genotypes (HLA-B51/HLA-B5)</td>
<td>23/24</td>
<td>0.52</td>
<td>19/20</td>
<td>0.63</td>
</tr>
<tr>
<td>Genotyping method (serological/molecular/NR)</td>
<td>25/6/16</td>
<td>0.80</td>
<td>18/5/16</td>
<td>0.06</td>
</tr>
<tr>
<td>Publication year</td>
<td>47</td>
<td>0.91</td>
<td>39</td>
<td>0.85</td>
</tr>
<tr>
<td>Publication language (English/other)</td>
<td>36/11</td>
<td>0.008</td>
<td>29/10</td>
<td>0.80</td>
</tr>
</tbody>
</table>

\(a\)ISG criteria; JBDRC criteria; other criteria or not stated. \(b\)Asia; Middle East/North Africa; Europe. NR: not reported; \(P_{\text{cov}}\): P-value for covariate in meta-regression.

**Fig. 3** Pooled frequencies of clinical BD features according to HLA-B51/B5 status derived from data sets combining 5–47 patient populations. For each clinical variable, percentages are given for HLA-B51/B5-positive vs HLA-B51/B5-negative subjects. Calculations used random-effects meta-analysis techniques. Primary analyses identified variables marked with an asterisk as having statistically significant increased or decreased RR of occurrence according to HLA-B51/B5 status.

[Graph showing pooled frequencies of clinical BD features stratified by HLA-B51/B5 status.]

The results of these analyses are shown in Table 2. Fig. 3 shows the pooled frequencies of the phenotypic characteristics stratified by HLA-B51/B5 status. The estimated proportions of HLA-B51/B5-positive cases in these 14 population pools ranged from 49.8% (95% CI 30.9%, 68.7%) (for the pseudofolliculitis meta-analysis) to 63.8% (95% CI 45.7%, 78.7%) (for the chest involvement meta-analysis).
Discussion

Herein we reported the findings of 14 comprehensive meta-analyses, which pooled between 5 and 47 individual study populations, to estimate the effect of HLA-B51/B5 on BD clinical features. The summary estimates support that HLA-B51/B5 carriage is associated with significant 7–13% relative increases of the prevalence of genital ulcers, ocular or skin involvement, and a significant 30% relative reduction on the prevalence of gastrointestinal involvement. A significant link between HLA-B51/B5 positivity and male sex was also found.

Current understanding is that both innate and adaptive immune responses are implicated in BD pathogenesis [111]. Neutrophils, the prototypic cells of the innate immune system, are key effectors of BD, as highlighted by skin hyperreactivity (pathergy reaction), neutrophil infiltrates seen in papulopustular skin lesions [112] and the demonstrated efficacy of the neutrophil function-inhibitor colchicine [113] on BD skin lesions and uveitis [114, 115].

Intervention of the adaptive immune system is supported by the association of BD with HLA-B51/B5, findings of an oligoclonal profile of the T-lymphocyte repertoire [116] and the efficacy of immunosuppressive therapy against some BD manifestations [117]. As is true for virtually all MHC–disease associations [111, 118], the mechanism by which HLA-B51 predisposes to BD remains elusive. The most compelling theory is that HLA-B51 presents autoantigens to T-suppressor lymphocytes and thereby activates the immune system.

The main finding of this study was that HLA-B51/B5 had only a modest phenotype-fostering effect in BD. It implies that the clinical pictures of HLA-B51/B5-positive and negative BD are virtually indistinguishable, and that genotyping of this allele cannot accurately predict the occurrence of specific organ or system manifestations. That HLA-B51/B5 was not significantly associated with the main markers of increased mortality, i.e. major vessel involvement [119, 120] or CNS disease [119, 121], indirectly suggests that this allele cannot serve as a mortality risk surrogate. In this study, the genetic effects were mainly assessed as generic organ or system involvements (e.g. eye involvement) and it cannot be excluded that HLA-B51/B5 positivity drives a particular pattern of organ or system involvement (e.g. posterior uveitis). Thus the majority of computed RR only marginally deviated from the line of identity, making it unlikely that HLA-B51/B5 has a numerically strong and clinically meaningful effect on a specific clinical manifestation. The observation that more variables were positively rather than negatively associated with HLA-B51/B5 could still suggest that BD carriers of this allele have more extensive disease.

Notwithstanding, the slight but significant frequency increases observed for genital ulcers, skin involvement and eye involvement among HLA-B51/B5 allele carriers merit consideration. These findings suggest that these manifestations, which form with oral ulcers the core symptoms of BD [102], have a shared pathogenetic pathway and favour the idea that HLA-B51/B5 has a permissive effect on their development. When adhering to the paradigm that cutaneous and ocular manifestations are essentially neutrophil induced, these results add support to previous observations of a connection between HLA-B51 positivity and neutrophil dysfunction. Although not uncontested [122], results of studies on mice and humans have suggested that HLA-B51 plays a role in neutrophil activation [123] and chemotaxis [50, 124]. In contrast, the protective role of HLA-B51/B5 on gastrointestinal disease is difficult to conceptualize. The hazy clinical line between gastrointestinal BD and other inflammatory bowel diseases, namely ulcerative colitis and Crohn’s disease [4], for which no relationship with HLA-B51/B5 exists [125], begs the question as to whether this finding mirrors some degree of diagnostic misclassification.

Another remarkable indication of this study is that HLA-B51/B5 carriage was more common in male BD patients. Similar observations were made previously [31, 52, 66, 75], and they also agree with the findings of another meta-analysis that showed that the strength of the HLA-B51/B5–BD relationship was positively correlated with the proportion of male cases included in individual studies [3]. It could be advanced that the link between HLA-B51/B5 and male sex is merely confounded by the previously reported evidence suggesting that male BD cases are more prone to eye involvement [66, 126–130]. However, the results of a study in which the HLA-B51–ocular disease relationship was stratified by sex suggested that the ocular disease-promoting effect of this allele is not restricted to males [54]. Moreover, as highlighted by the estimate that 59% were males in our corresponding meta-analysis data set, a male predominance in BD has been seen in many geographical areas [131] and could provide indirect support for HLA-B51/B5 conferring enhanced BD risk to men.

Whether this sex-specific effect accounts for genetic or non-genetic epistasis is elusive. It was reported that testosterone levels correlated with markers of neutrophil activation in BD patients [132], and collectively these observations could indicate that male hormones act in concert with HLA-B51/B5, contributing to neutrophil dysfunction. It is also worth noting that, in our meta-regression analysis, the HLA-B51/B5 relationship with male sex was only found in studies conducted in Asia and Europe, but not the Middle East or North Africa.

The meta-analysis data sets enabled computation of frequency estimates for BD manifestations within large case populations. These pooled estimates could reflect a truer picture than those observed in individual hospital series, which are vulnerable to selection bias. As it was advanced that there are geographic variations of BD clinical expression [131], these numbers need to be interpreted with the caveat in mind that they combined distinct international locations. BD cases harbouring HLA-B51/B5 ranged between 50% and 72% across the 14 meta-analysis data sets, and the consistency of these estimates with previously established figures [3] supports the representativeness of our assembled data sets.

A potential shortcoming of our analyses is the possible between-study differences in the definitions used for...
organ and system involvements. In addition, publication bias likely occurred in the meta-analyses for sex and eye involvement, and selective reporting of the outcomes in the primary cohorts cannot be ruled out. Thus the possibility that our findings were substantially flawed by these aspects is tempered by the, at best, small statistical between-study heterogeneity in most of the generated meta-analyses, and the results remained robust in sensitivity analyses restricted to the larger studies. In addition, meta-regression analyses suggested that several study characteristics possibly affected the strengths of association between HLA-B51/B5 and eye involvement, which could raise concern about the magnitude of the estimated risk of eye involvement with allele carriage. Another potential drawback of our study is that, for clinical features that are either uncommon or with only small numbers of identified informative studies, statistical power may have been insufficient.

In conclusion, these quantitative syntheses of the HLA-B51/B5 impact on clinical BD features provide evidence that HLA-B51/B5 positivity has only modest BD phenotype-modifying effect and emphasize the potential interplay between this allele and neutrophil dysfunction. This study’s findings may contribute to improving our understanding of the clinical continuum of BD presentations and their pathophysiologies.

Rheumatology key messages

- HLA-B51/B5 is moderately associated with genital ulcers, eye and skin involvement in BD.
- HLA-B51/B5 carriage is slightly higher in male BD.
- In BD, HLA-B51/B5 may exert its effect via a neutrophilic pathway.

Acknowledgements

We are grateful to the following physicians and researchers for their help in the selection of relevant articles: Z. Alekberova (Moscow, Russia), A. M. Chamberlain (Leeds, UK), Y. M. Chung (Taipei, Taiwan/China), S. Hirohata (Tokyo, Japan), I. Köttter (Tübingen, Germany), I. Krause (Ptech Tikva, Israel), S. Ohno (Sapporo, Japan), P. Pistetti-Pezzi (Rome, Italy), N. Pipitone (Reggio Emilia, Italy), H. Yazici, S. Yurdakul (Istanbul, Turkey) and C. C. Zouboulis (Dessau, Germany).

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

References


