Chapter 8

Butyrophilin-like 2 in pulmonary sarcoidosis: a factor for susceptibility and progression?

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Abstract

Background
The aims of this study were to assess the association of \textit{BTNL2} G16071A with the course of pulmonary sarcoidosis and verify association with disease predisposition. In addition, the linkage between \textit{BTNL2} G16071A and certain HLA-DRB1/DQB1 types was investigated.

Methods
In a retrospective case-control study \textit{BTNL2} G16071A, HLA-DQB1 and DRB1 were typed in 632 sarcoidosis patients. These patients were classified into 304 patients with persistent and 328 patients with non-persistent sarcoidosis.

Results
The \textit{BTNL2} 16071A variant allele was significantly more often present in patients with persistent disease (92.4%; 281/304) compared with patients demonstrating a non-persistent course (86.6%; 284/328); odds ratio (OR)=1.89 with 95% confidence interval (CI 95%): 1.11-3.22.
Furthermore, \textit{BTNL2} 16071A variant allele carriers have an increased risk (OR=1.85, CI 95%: 1.19-2.88) to develop sarcoidosis. Moreover, the strong linkage between variant allele and HLA-DRB1*15 presence (OR=8.43, CI 95%: 3.02-23.5) was confirmed.

Conclusion
The presence of a \textit{BTNL2} G16071A variant allele almost doubles the risk of progressing to persistent pulmonary sarcoidosis, besides increasing the risk to develop sarcoidosis. Presumably, these increased risks are due to the strong linkage between \textit{BTNL2} G16071A and DRB1*15. The choice between determining \textit{BTNL2} G16071A SNP or the HLA-DRB1 type depends on the ability and/or availability to perform either test.
Introduction

Sarcoidosis is a multisystem granulomatous disorder of which clinical presentation and outcome vary considerably.\(^1\)\(^-\)\(^3\) The assumption that genes contribute to the etiology of sarcoidosis comes from the observation that prevalence and incidence rates of sarcoidosis are different between ethnic groups and that the disease tends to cluster in families. Next to family clustering, disease clustering in time and place, often with co-workers, was also noted.\(^4\) Therefore, shared common environmental exposures must be considered as well. However, the etiology may not prove to be a single, known exposure, and the confirmation of interactions of exposure with genetic predispositions would have important implications for the understanding of immune responses as well as the pathogenesis of sarcoidosis.\(^4\)

In previous studies the association between single nucleotide polymorphisms (SNPs) in the butyrophilin-like 2 (\textit{BTNL2}) gene and the risk to develop sarcoidosis has been shown.\(^5\)\(^-\)\(^8\) The \textit{BTNL2} G16071A (rs2076530) polymorphism showed the strongest association with sarcoidosis.\(^6\) Arnett et al. found that the functional \textit{BTNL2} reduces proliferation and cytokine production from activated T-cells, suggesting a role for \textit{BTNL2} as a negative co-stimulatory molecule with implications for inflammatory disease.\(^9\) The A-allele of the \textit{BTNL2} G16071A polymorphism causes a premature truncation of the protein, disrupting insertion in the cell membrane, a necessary process for downregulating activated T-cells (Th1).\(^5\)\(^,\)\(^10\) The truncated protein increases the risk of developing sarcoidosis independent of HLA-DRB1 risk alleles.\(^5\)\(^,\)\(^6\)

However, a possible association with the prognosis of sarcoidosis was not investigated. Until now an association with chronic sarcoidosis only has been suggested, because of an established strong linkage of the \textit{BTNL2} 16071A variant allele with HLA-DRB1*15 (DRB1*15).\(^6\) Association of sarcoidosis and class I and II HLA antigens is well known and several studies established the risk of progression of sarcoidosis and presence or absence of specific HLA-types.\(^11\)\(^-\)\(^15\) In addition, DRB1*15 and the DQB1*0602/DRB1*1501 haplotype in particular, was associated with more severe sarcoidosis, whereas DRB1*03 has been associated with good prognosis.\(^11\)\(^,\)\(^13\)\(^,\)\(^16\)\(^,\)\(^17\)

Pulmonary disease is the most common manifestation of sarcoidosis and pulmonary symptoms are the most common reason for treatment.\(^1\)\(^,\)\(^18\) Numerous studies have confirmed the utility of chest X-ray (CXR) stages as a prognostic guide.\(^1\) The presence of hilar adenopathy alone (CXR stage I) is usually associated with good prognosis.\(^1\)\(^,\)\(^11\)\(^,\)\(^19\) By contrast, presence of parenchymal infiltrates (CXR stages II-IV) is associated with chronic disease.\(^1\)\(^,\)\(^19\) Spontaneous remissions occur in 55-90\% of patients with stage I, 40-70\% of patients with stage II, 10-20\% of patients with stage III, and in 0\% of patients with stage IV disease.\(^1\) Because of recognized value, CXR staging
was used in several studies to categorize sarcoidosis patients into subgroups with different pulmonary manifestations.\textsuperscript{1,20,21}

The aims of this study were to assess the association of \textit{BTN}L2 G16071A with the course of pulmonary sarcoidosis and to verify the association with disease predisposition. In addition, the linkage between \textit{BTN}L2 G16071A and certain HLA-DRB1/DQB1 types was investigated.

Materials and methods

Patients

From January 2000 to July 2008, 632 consecutive Dutch Caucasian sarcoidosis patients, attending the outpatient referral clinic of the ield care center of the Department of Respiratory Medicine of the Maastricht University Medical Centre (MUMC) The Netherlands, were included in this study. Time between diagnosis or inclusion and end of follow-up for all patients was at least two years. Diagnosis was in 71\% of cases based on a positive biopsy (lung, skin, lymph node or liver). In patients with typical features of Löfgren’s syndrome and characteristic features of bronchoalveolar lavage (BAL) fluid analysis results (including lymphocytosis), no biopsy was obtained.\textsuperscript{22,23} This policy is consistent with the World Association of Sarcoidosis and Other Granulomatous diseases (WASOG) guidelines.\textsuperscript{1}

A control group consisting of 1474 local, healthy, unrelated Dutch Caucasian volunteers, hospital workers, and blood or stem cell donors (age: 48.8±10.3 (21-86); 48.6\% female) was used for HLA typing. Out of this control group 200 samples were randomly selected and typed for the \textit{BTN}L2 SNP.

The study was performed in accordance with the Declaration of Helsinki and its amendments. The protocol was approved by the Medical Ethics Board of the MUMC. Written informed consent for participation in this study was obtained from all subjects.

Collection of clinical data

At inclusion forced vital capacity (FVC) and forced expiratory volume in one second (FEV\textsubscript{1}) were measured with a pneumotachograph (Masterlab, Jaeger, Würzburg, Germany). The diffusing capacity for carbon monoxide (DLCO) was measured by the single-breath method (Masterlab). Values were expressed as a percentage of predicted values. Missing values for DLCO/FEV\textsubscript{1}/FVC were 17/21/26, respectively. The cut-off value for the DLCO, FEV\textsubscript{1}, and FVC was <80\% of predicted (≥80\% is normal).\textsuperscript{24} Respiratory functional impairment (RFI) was defined as DLCO<80\%, FVC<80\% or FEV1<80\% (percentage of
predicted). Patients without RFI were those for whom all three indices were normal (≥80%).

Clinical course of patients with sarcoidosis was defined using changes in CXR stage during follow-up. All CXRs were graded by a single experienced observer, who was not aware of the clinical data. Five stages of radiographical abnormality were recognized: stage 0 (normal CXR), stage I (bilateral hilar lymphadenopathy [BHL]), stage II (BHL and parenchymal abnormalities), stage III (parenchymal abnormalities without BHL), and stage IV (end stage lung fibrosis).

For the analysis with respect to prognosis, patients were categorized into groups with persistent and non-persistent sarcoidosis. Persistent disease was defined as worsening of the CXR stage to stage II or higher, or remaining at CXR stage II or III, at least two years after diagnosis. Non-persistent disease cases were those who remained at or regressed to stage 0 or I.

In addition to the categorization into persistent/non-persistent sarcoidosis, CXR stages were used combined with lung function test results to classify the patients into groups with and without progressive disease. No progression was defined as remaining at stage 0, I, II or III or regressing to stage 0 or I. Progression was defined as worsening of the CXR stage or remaining at stage I, II or III in combination with RFI.

BTNL2 and HLA typing

DNA isolation

DNA was obtained using venous EDTA or heparin anti-coagulated blood and isolated with a High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany), or with QIA-AMP kits (Qiagen, Westburg, Leusden, The Netherlands) according to the manufacturer’s instructions.

BTNL2

Patients and controls were genotyped for the BTNL2 G16071A (rs2076530) polymorphism using a real-time PCR Fluorescence Resonance Energy Transfer (FRET) assay (TIB MOLBIOL, Berlin, Germany) on the LightCycler® (Roche Diagnostics).

HLA

Low resolution typing of HLA-DRB1 and DQB1 was obtained by Luminex reverse SSO, using bead kits from One Lambda (One Lambda, Bethesda, USA) or by PCR-SSP, using 45 in-house primer mixes as described previously. High resolution typing of DRB1 and DQB1 was obtained by sequencing exon 2 and if needed exon 3, as previously described. Either solid phase
sequencing was used as described, or cycle sequencing was performed using Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, USA). In cases of ambiguous typing, allele specific sequencing was performed, using suitable allele specific amplification primers in combination with adjusted sequencing primers as described.

Statistical analysis
The chi-square test was used to test for statistical significant differences between groups. Odds ratios (OR) with 95% confidence intervals (CI) were derived from cross tables to evaluate strength of associations. Multivariate logistic regression models were used to adjust for differences in baseline characteristics (sex, smoking, and corticosteroid use) between compared groups. A p<0.05 (two sided) was considered to indicate statistical significance. Deviations from the Hardy–Weinberg equilibrium were analysed using the chi-square test. Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, USA) for Windows.

Results
Patient characteristics of the total sarcoidosis population and of the subgroups with persistent and non-persistent sarcoidosis are summarized in Table 8.1. Patients with persistent disease more often were male, smoked less often and were more frequently treated with corticosteroids. At the time of diagnosis, the percentage with CXR stage 0 or I was much lower in patients with persistent disease than in the patients with non-persistent disease. Persistent disease was also associated with lower mean values of DCLO, FEV₁ and FVC at diagnosis.

In Table 8.2 an overview of \textit{BTNL2} G16071A allele and HLA-DRB1*15 phenotype frequencies found in our entire sarcoidosis patient group (n=632) and in sarcoidosis patients with persistent and non-persistent disease is shown. The local healthy Dutch Caucasian control population (n=1474 for HLA and n=200 randomly selected out of the total 1474 for \textit{BTNL2} G16071A) establishing that our ‘local’ healthy population did not differ from historic controls from literature with the same ethnicity regarding the \textit{BTNL2}), and several patient and control populations taken from the literature, are also listed for comparison.
Table 8.1 Patient characteristics for the total sarcoidosis population and sorted by disease persistence.

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>Non-persistent</th>
<th>Persistent</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>283 (44.8)</td>
<td>177 (54.0)</td>
<td>106 (34.9)</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>349 (55.2)</td>
<td>151 (46.0)</td>
<td>198 (65.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>age at diagnosis</strong></td>
<td>yr±SD (range)</td>
<td>40.2±11.7 (12-84)</td>
<td>40.5±12.6 (17-84)</td>
<td>39.9±10.7 (12-76)</td>
</tr>
<tr>
<td>&lt; 40 yr</td>
<td>356 (56.3)</td>
<td>185 (56.4)</td>
<td>171 (56.2)</td>
<td></td>
</tr>
<tr>
<td>≥ 40 yr</td>
<td>276 (43.7)</td>
<td>143 (43.6)</td>
<td>133 (43.8)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>562 (88.9)</td>
<td>283 (86.3)</td>
<td>279 (91.8)</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>347 (54.9)</td>
<td>141 (43.0)</td>
<td>206 (67.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>corticosteroid use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>285 (45.1)</td>
<td>187 (57.0)</td>
<td>98 (32.2)</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>347 (54.9)</td>
<td>141 (43.0)</td>
<td>206 (67.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>CXR at diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>66 (10.4)</td>
<td>66 (20.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>186 (29.4)</td>
<td>172 (52.5)</td>
<td>14 (4.6)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>245 (38.8)</td>
<td>66 (20.1)</td>
<td>179 (58.9)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>135 (21.4)</td>
<td>24 (7.3)</td>
<td>111 (36.5)</td>
<td></td>
</tr>
<tr>
<td><strong>CXR at follow-up</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>0</td>
<td>282 (44.6)</td>
<td>282 (86.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>46 (7.3)</td>
<td>46 (14.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>141 (22.3)</td>
<td>0</td>
<td>141 (46.4)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>76 (12.0)</td>
<td>0</td>
<td>76 (25.0)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>87 (13.8)</td>
<td>0</td>
<td>87 (28.6)</td>
<td>&lt;0.001c</td>
</tr>
</tbody>
</table>

**DLCO**

- mean±SD (range) 81.4±17.3 (23-129) 87.0±15.0 (37-129) 75.4±17.5 (23-121) <0.001c
- ≥80% 353 (57.4) 235 (73.9) 118 (39.7) <0.001
- <80% 262 (42.6) 83 (26.1) 179 (60.3) <0.001

**FEV<sub>1</sub>**

- mean±SD (range) 90.2±21.5 (23-140) 100.5±15.3 (54-140) 79.0±21.8 (23-128) <0.001
- ≥80% 448 (73.3) 294 (92.5) 154 (52.6) <0.001
- <80% 163 (26.7) 24 (7.5) 139 (47.4) <0.001

**FVC**

- mean±SD (range) 99.0±19.1 (25-152) 106.6±15.3 (66-152) 90.7±19.4 (25-148) <0.001
- ≥80% 516 (85.1) 303 (95.6) 213 (73.7) <0.001
- <80% 90 (14.9) 14 (4.4) 76 (26.3) <0.001

* non-persistent versus persistent. b CXR 0+I versus II+III. c CXR IV versus 0+I+II+III. d % of predicted (<80% is abnormal). n=number, yr=years, NS=not significant, SD=standard deviation, CXR=chest X-ray, DLCO=diffusing capacity of carbon monoxide, FEV<sub>1</sub>=forced expiratory volume in one second, FVC=forced vital capacity. Missing: 17/21/26 for DLCO/FEV<sub>1</sub>/FVC, respectively. All values presented are absolute numbers with percentages in parentheses unless otherwise specified.

In the healthy controls the proportions of persons without or with a **BTNL2** 16071A variant allele were 18.0% (GG) and 82.0% (44.0% GA and 38.0% AA), respectively (chi²=1.39, p=0.24). These findings were in accordance with the Hardy-Weinberg equilibrium.
Table 8.2 Frequencies of BTNL2 G16071A and HLA-DRB1*15 sarcoidosis patients and controls.

<table>
<thead>
<tr>
<th>Population</th>
<th>number</th>
<th>BTNL2 G-allele %</th>
<th>BTNL2 A-allele %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoidosis patients</td>
<td>632</td>
<td>33.9</td>
<td>66.1</td>
</tr>
<tr>
<td>Non-persistent sarcoidosis</td>
<td>328</td>
<td>36.0</td>
<td>64.0</td>
</tr>
<tr>
<td>Persistent sarcoidosis</td>
<td>304</td>
<td>31.6</td>
<td>68.4</td>
</tr>
<tr>
<td>UK+Dutch patients(^{45})</td>
<td>288</td>
<td>33.2</td>
<td>66.8</td>
</tr>
<tr>
<td>German patients(^{3})</td>
<td>904</td>
<td>31.4</td>
<td>68.6</td>
</tr>
<tr>
<td>German patients(^{7})</td>
<td>210</td>
<td>30.7</td>
<td>69.3</td>
</tr>
<tr>
<td>Local controls</td>
<td>200</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Dutch controls(^{41})</td>
<td>351</td>
<td>41.2</td>
<td>58.8</td>
</tr>
<tr>
<td>German controls(^{8})</td>
<td>427</td>
<td>42.7</td>
<td>57.3</td>
</tr>
<tr>
<td>German controls(^{2})</td>
<td>202</td>
<td>38.1</td>
<td>61.9</td>
</tr>
<tr>
<td>UK+Dutch controls(^{45})</td>
<td>446</td>
<td>42.6</td>
<td>57.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population</th>
<th>HLA-DRB1*15 neg %</th>
<th>HLA-DRB1*15 pos %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoidosis patients</td>
<td>632</td>
<td>31.8</td>
</tr>
<tr>
<td>Non-persistent sarcoidosis</td>
<td>328</td>
<td>25.9</td>
</tr>
<tr>
<td>Persistent sarcoidosis</td>
<td>304</td>
<td>38.2</td>
</tr>
<tr>
<td>Local controls</td>
<td>1474</td>
<td>22.9</td>
</tr>
<tr>
<td>Dutch controls(^{46})</td>
<td>207</td>
<td>30.9</td>
</tr>
<tr>
<td>Dutch controls(^{47})</td>
<td>2400</td>
<td>26.0</td>
</tr>
<tr>
<td>Dutch controls(^{48})</td>
<td>700</td>
<td>23.8</td>
</tr>
<tr>
<td>UK controls(^{49})</td>
<td>537</td>
<td>20.9</td>
</tr>
</tbody>
</table>

Table 8.3 shows the distributions of the studied BTNL2 polymorphism and HLA types in healthy controls, the total sarcoidosis population, and subgroups with and without persistent disease or progression. The BTNL2 G16071A polymorphism occurs more frequently in the total sarcoidosis population than in the healthy local controls (OR=1.85; p=0.007).

The BTNL2 16071A variant allele was more often present in patients with persistent disease when compared with patients having non-persistent disease (OR=1.89; p=0.02). After multivariate correction for sex, smoking, and corticosteroid use the OR remained similar (OR=1.84, CI 95%: 1.06-3.21; p=0.03). The ORs for the subgroups no progression and progression were similar to those with and without persistent disease (Table 8.3).

Significant differences with respect to the HLA types DRB1*03 and DRB1*15 (with or without DQB1*06) were also observed. For the DRB1*15 a significantly larger proportion of patients with persistent sarcoidosis was positive, 38.2% versus 25.9% in the non-persistent group (OR=1.76; p=0.001). The presence of DRB1*15 in combination with DQB1*06 (15Q6 haplotype) was also associated with an increased risk of poor prognosis: OR of 1.63 (p=0.006). From all but 60 patients high resolution typing of the DQB1*06 allele was available, and presence of the DQB1*0602 allele was
associated with poor prognosis: OR=2.36, CI 95%: 1.59-3.49; p<0.001 (data not shown).

Table 8.3 Distributions for the controls, total sarcoidosis population, and sorted by disease persistence.

<table>
<thead>
<tr>
<th>Type</th>
<th>Controls n=200/1474</th>
<th>Total population (range)</th>
<th>Odds ratio</th>
<th>Non-persistent n=328</th>
<th>Persistent n=304</th>
<th>Odds ratio</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTNL 71 GG</td>
<td>36 (18.0)</td>
<td>67 (10.6)</td>
<td>44 (13.4)</td>
<td>23 (7.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>88 (44.0)</td>
<td>294 (46.5)</td>
<td>148 (45.1)</td>
<td>146 (48.0)</td>
<td>1.89 (1.11-3.22)</td>
<td>0.02**</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>76 (38.0)</td>
<td>271 (42.9)</td>
<td>136 (41.5)</td>
<td>135 (44.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*03 neg</td>
<td>1140 (77.3)</td>
<td>476 (75.3)</td>
<td>215 (65.5)</td>
<td>261 (85.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pos</td>
<td>334 (22.7)</td>
<td>156 (24.7)</td>
<td>113 (34.5)</td>
<td>43 (14.1)</td>
<td>0.31 (0.21-0.46)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>DRB1*15 neg</td>
<td>1136 (77.1)</td>
<td>431 (68.2)</td>
<td>243 (74.1)</td>
<td>188 (61.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pos</td>
<td>338 (22.9)</td>
<td>201 (31.8)</td>
<td>85 (25.9)</td>
<td>116 (38.2)</td>
<td>1.76 (1.26-2.47)</td>
<td>0.001</td>
<td></td>
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<tr>
<td>15Q6</td>
<td>1145 (77.7)</td>
<td>441 (69.8)</td>
<td>245 (74.7)</td>
<td>196 (64.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neg</td>
<td>329 (22.3)</td>
<td>191 (30.2)</td>
<td>83 (25.3)</td>
<td>108 (35.5)</td>
<td>1.63 (1.16-2.9)</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

a controls versus total population. b non-persistent versus persistent. c 200 healthy, unrelated, Dutch Caucasian volunteers for the BTNL2 and 1474 for the HLA typing. d no variant allele versus variant allele. NS=not significant, BTNL 71=BTNL2 G16071A (rs2076530), DRB1*03=HLA-DRB1*03, DRB1*15=HLA-DRB1*15, 15Q6=HLA-DQB1*06/DRB1*15 haplotype. All values presented are absolute numbers with percentages in parentheses unless otherwise specified.

Within the healthy Dutch Caucasian controls who were DRB1*15 positive, DQB1*06 was absent in 0.6% (9/1474). In the DRB1*15 positive sarcoidosis patients with non-persistent disease a comparable percentage without a DQB1*06 (0.6%; 2/328) was found, whereas in the subgroup with persistent disease 2.6% (8/304) of the patients possessed a DRB1*15 type without bearing a DQB1*06. Within the DRB1*15 bearing group, DQB1*06 showed a negative association with progressive disease, but due to the small numbers the confidence interval around the odds ratio is very wide. (OR=0.18, CI 95%: 0.02-1.54; p=0.14). These findings indicate a trend towards an effect of DRB1*15 on the course of sarcoidosis independent of DQB1*06. In contrast to the DRB1*15 results, the presence of a DRB1*03 allele was associated with a good prognosis (OR=0.31; p<0.001).

The presence of DRB1*15 was strongly associated with the presence of a BTNL2 16071A variant allele: OR=8.43 (CI 95%: 3.02-23.5; p<0.001). Within the subgroup of patients who were positive for the DRB1*15 (n=201) only 2% (n=4) did not possess a BTNL2 16071A variant allele, compared with 14.6% (n=63) within the subgroup of DRB1*15 negative patients. Possessing neither a DRB1*15 nor the BTNL2 G16071A variant allele compared with both the DRB1*15 and a BTNL2 16071A variant allele being present in patients displaying persistent disease, resulted in OR=2.61 (CI 95%: 1.45-4.72; p=0.001) for the DRB1*15/BNL2 positive haplotype. The OR associated with presence of the BTNL2 G16071A in absence of DRB1*15 for persistence is
1.53 (CI 95%: 0.88-2.67; \( p=0.17 \)) and this OR indicates that the effect of the 
BTNL2 is independent of DRB1*15.
The OR obtained for the combined presence of the 15Q6 haplotype and the 
\( {BTNL2} \ 16071A \) variant allele were comparable to the results of the DRB1*15 
and \( {BTNL2} \ 16071A \) variant allele: OR=7.79 (CI 95%: 2.79-21.7; \( p<0.001 \)).
The observed association between the presence of DRB1*03 and \( {BTNL2} \ 16071A \) 
A-allele presence was in the opposite direction: OR=0.08, CI 95\%: 0.02-0.32; 
\( p<0.001 \). Of the subgroup of patients who were positive for the DRB1*03 
(n=156) only 1.3% (n=2) did not possess a \( {BTNL2} \ 16071A \) variant allele, 
compared with 13.7\% (n=65) within the subgroup of DRB1*03 negative 
patients.

Discussion

This study is the first that demonstrates that the presence of the \( {BTNL2} \) 
16071A variant allele increases the risk of progressing to more severe and 
persistent pulmonary sarcoidosis. Previously, an association between the 
presence of the \( {BTNL2} \ 16071A \) variant allele and a susceptibility towards the 
chronic form of sarcoidosis was suggested and an increase in A-allele frequency 
in non-Löfgren patients was observed.\(^7,29\) However, the A-allele was not 
considered a risk factor in the progressing of pulmonary sarcoidosis. 
Furthermore, it is confirmed in accordance with others, that the \( {BTNL2} \ 16071A \) 
variant allele presence almost doubles the risk of developing sarcoidosis. In 
addition, the strong linkage of the \( {BTNL2} \ G16071A \) with HLA-DRB1*15 is 
confirmed. This explains why DRB1*15 bearing also is found to be a risk factor 
for disease susceptibility as well as for disease course.

It is well known that the outcome of sarcoidosis varies considerably.\(^30\) 
Inconsistency exists on how to define severity. The course of sarcoidosis is 
mainly monitored by assessing clinical features and using auxiliary diagnostic 
tests. In the past decade, insight has been provided into the genetic risk for 
sarcoidosis and how the genetic make-up of a patient determines the clinical 
presentation and outcome. Two more provocative studies in sarcoidosis include 
the recognition of the \( {BTNL2} \) gene as a candidate sarcoidosis susceptibility 
gene\(^5,6\) and the identification of mycobacterial catalase-peroxidase as a 
potential sarcoidosis antigen\(^31\) that drives granuloma formation.\(^32\) The \( {BTNL2} \) 
gene is located close to and in linkage with HLA-DRB1, which in turn is 
implicated in the etiology of sarcoidosis.\(^21,33-35\) The \( {BTNL2} \ G16071A \) 
polymorphism in particular has been linked with an increased susceptibility risk 
for developing sarcoidosis.\(^5-7\) However, other than the suggestion that 
susceptibility might be preferential towards the chronic form of sarcoidosis\(^7\),
there were no studies done exploring the possibility that \textit{BTNL2} G16071A could also be associated with an increased risk of progression. In this study it was found that possessing the A-allele almost doubles (OR=1.89, CI 95%: 1.11-3.22) the risk of a persistent course of sarcoidosis. When also using lung function test results to categorize the sarcoidosis patients into groups with and without progression the OR remains similar (OR=1.75, CI 95%: 1.01-3.04). Next to other progression markers, such as DRB1*03 or DRB1*15 phenotypes \cite{11,13,36} or the \textit{TNF-\alpha} G-308A polymorphism \cite{14,37,38}, one could argue that \textit{BTNL2} G16071A does not add much. However, especially in families with sarcoidosis patients, this SNP could provide information about the risk of developing sarcoidosis and when present, about the course of the disease. Moreover, as was found in a study by Coudurier et al. examining a family of sarcoidosis patients, the A/A genotype of \textit{BTNL2} G16071A can be defined as a putative prognosis and/or predictive factor of recurrent and severe sarcoidosis. \cite{8} Therefore, genotyping before developing sarcoidosis in persons with a relative with the disease, might give additional information about disease risk and course, and can facilitate disease management. Prospective family-based studies should be performed to confirm this.

In our study the A-allele of the \textit{BTNL2} G16071A also appeared to increase the risk of developing sarcoidosis almost twofold, in agreement with results found by Valentonyte et al. \cite{5} No significant association was established in a study performed on other diseases, despite the fact that these were also Th1 dominated granulomatous diseases similar to sarcoidosis, and a role for MHC class II (HLA-DR/DQ) genes was found in these conditions. \cite{35,39-44} A possible explanation for the lack of a significant association between \textit{BTNL2} and sarcoidosis in general might be the result of differences in the patient and healthy volunteer populations studied. This once again draws the attention to the importance of selection and description of the used populations in genetic association studies. \cite{45,46} In other studies the association between \textit{BTNL2} and HLA-DRB1/DQB1 was found, but because of the strong linkage disequilibrium attributed to HLA-DR/DQ haplotypes, \cite{35,47} Indeed, several previous studies in other diseases have convincingly and consistently shown association between HLA-DRB1 (more specifically the 15Q6 haplotype or DRB1*15) and disease susceptibility. \cite{48-50}

In the present study, the strong linkage disequilibrium for the association between DRB1*15 and \textit{BTNL2} G16071A was confirmed (OR=8.43, CI 95%: 3.02-23.5). Moreover, of the DRB1*15 bearing sarcoidosis patients only four did not possess a \textit{BTNL2} 16071A variant allele. Comparing the healthy Dutch Caucasian controls (n=1474) with the total sarcoidosis population (n=632) for the DRB1*15 resulted in a significant difference indicating an increased risk for
developing sarcoidosis when DRB1*15 positive. In addition, an association between presence of DRB1*15 and persistent sarcoidosis (OR=1.76, CI 95%: 1.26-2.47) was established. This was in agreement with results from previous studies.\textsuperscript{5,6,13} This indicates that DRB1*15 positivity can be considered as an important factor in both the susceptibility to sarcoidosis and the severity of the disease, comparable with the presence of a \textit{BTNL2} 16071A variant allele. In addition, the \textit{BTNL2} 16071A/DRB1*15 positive haplotype increases the risk of a persistent course of pulmonary sarcoidosis almost threefold. However, the possibility remains that due to the strong linkage, other functional variations on this \textit{BTNL2}/DRB1*15 haplotype may be the true or additional causal determinants.\textsuperscript{35} Nevertheless, the strong linkage disequilibrium between \textit{BTNL2} and HLA-DR/DQ requires that \textit{BTNL2} G16071A should be considered relevant to any immune-related disease associated with HLA-DR/DQ.\textsuperscript{35}

Previously, an association of the 15Q6 haplotype with the severity of sarcoidosis was found.\textsuperscript{13} However, because of the rather limited number of patients (n=156) and controls (n=418) and the strong linkage disequilibrium between DRB1*15 and DQB1*06, it could not be concluded whether the association was with either DRB1*15 or with DQB1*06.\textsuperscript{13} In the present study, with an extended number of patients and controls, it was found that the number of DRB1*15 bearing individuals without DQB1*06 was nine out of 1474 for the healthy controls and 10 out of 632 for the patients. Furthermore, it was two out of 328 out of the non-persistent group and eight out of 304 out of the persistent group. Together with a higher OR observed for the DRB1*15, this implicates that typing for HLA-DRB1 and HLA-DQB1 to ascertain the 15Q6 haplotype does not give any extra information, and that typing for DRB1 is sufficient.

In conclusion, the presence of a \textit{BTNL2} 16071A variant allele was found to be associated with an almost twofold increased risk of progressing to more severe and persistent pulmonary sarcoidosis in Caucasians. Furthermore, the predisposition to develop sarcoidosis was confirmed, as well as the strong linkage between the \textit{BTNL2} 16071A variant allele and DRB1*15 positivity. It also became apparent that typing for DRB1 is sufficient because of the lack of additional information obtained by typing the DQB1*06, to establish the 15Q6 haplotype. Whether or not to determine the DRB1 type or test the \textit{BTNL2} G16071A SNP therefore, depends on the ability and/or availability to perform either test. Additional research will be necessary to explore the role of these findings in the clinical management of sarcoidosis patients.
References


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