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Eur Respir J 2013; 42: 547–550 | DOI: 10.1183/09031936.00064713 | Copyright ©ERS 2013

Major histocompatibility complex class II and *BTNL2* associations in sarcoidosis

To the Editor:

Sarcoidosis is a multiple organ immune-mediated disease of unknown aetiology. Identified genetic risk factors within the major histocompatibility complex (MHC), such as *butyrophilin-like* (*BTNL)2*, human leukocyte antigen (*HLA)-DRB1*03* and *HLA-DRB1*15*, and protective factors, such as *HLA-DRB1*01*, are found in many populations [1, 2]. The vast majority of sarcoidosis patients have a favourable prognosis, but approximately 20% develop a chronic, disabling disease [3].

Chronic beryllium disease (CBD) and chronic sarcoidosis share similarities as granulomatous diseases and they are pathologically indistinguishable from each other. CBD has been associated with *HLA-DPB1*02:01*, especially with a glutamic acid residue at position 69 (Glu69) [4]. A functional splice-site polymorphism rs2076530 within the *BTNL2* gene has been suggested to predispose to sarcoidosis [5]. However, previous studies have shown conflicting results as to whether *HLA-DPB1* also predisposes to sarcoidosis, and whether the *BTNL2* association is a result of linkage disequilibrium with *HLA-DRB1* [6, 7].

The main objective of this study was to evaluate the *HLA-DPB1* polymorphisms and the *BTNL2* splice-site variant in Finnish patients suffering from sarcoidosis followed-up for 5–15 years and clinically categorised into subgroups based on disease prognosis. In addition, we constructed haplotypes containing MHC class II genes (*HLA-DRB1* and *-DPB1*) and rs2076530, and studied the influence of MHC markers and their combinations on disease susceptibility.

We examined a total of 188 patients with verified pulmonary sarcoidosis. The patients were divided into those with a disease resolved within 2 years (n=90) and those with persisting activity at that time point (n=98). The control population consisted of 150 healthy subjects representing the Finnish population. The characteristics of all have been previously reported [8]. All patients and controls gave their written informed consent to participate in the study.

Subjects were typed for *HLA-DPB1* (Invitrogen, Life Technologies, Carlsbad, CA, USA or Olerup SSP AB, Stockholm, Sweden) and rs2076530 (Sequenom, San Diego, CA, USA). In haplotype and linkage disequilibrium analysis the previously published *HLA-DRB1* alleles of the subjects were utilised [8].

Molecular analyses of MHC genes were performed using published protocols [8]. All comparisons were made between four different dichotomous outcome variables: all sarcoidosis patients *versus* controls; patients with resolved disease *versus* controls; patients with persistent disease *versus* controls; and disease prognosis was studied by comparing patients with resolved disease *versus* patients with persistent disease.

Table 1 provides a summary of the analyses. *HLA-DPB1* and rs2076530 loci were in Hardy–Weinberg equilibrium in both cases (*HLA-DPB1*: p=0.17; *BTNL2* rs2076530: p=0.74) and controls (*HLA-DPB1*: p=0.24; *BTNL2* rs2076530: p=0.86) measured directly by the exact test using the Markov-chain approach.

TABLE 1 Influence of significant major histocompatibility complex (MHC) markers, rs2076530 (A), *HLA-DRB1* alleles[#] and *HLA-DPB1*04:02*, on sarcoidosis

	Sarcoidosis *versus* controls			Resolved *versus* controls			Persistent *versus* controls			Resolved *versus* persistent		
	%	p-value	OR (95% CI)	%	p-value	OR (95% CI)	%	p-value	OR (95% CI)	%	p-value	OR (95% CI)
Influence of independent MHC markers on disease susceptibility[¶]												
*DRB1*03:01* pos[#]					0.002	2.8 (1.47–5.22)					0.005	2.8 (1.37–5.50)
*DRB1*15:01* pos[#]		0.036	1.7 (1.03–2.63)		0.001	2.7 (1.49–4.93)						
*DPB1*04:02* neg		0.007	2.0 (1.20–3.17)					0.004	2.4 (1.32–4.39)			
*DRB1*04:01-DPB1*04:01* pos					0.006	3.4 (1.42–8.24)					0.027	3.1 (1.14–8.65)
Influence of marker combinations on disease susceptibility[+]												
*DPB1*04:02* neg, rs2076530 (A) pos	72 *versus* 53	<0.001	2.3 (1.44–3.57)				79 *versus* 53	<0.001	3.2 (1.80–5.73)	65 *versus* 79	0.041	0.5 (0.27–0.98)
*DRB1*01:01* neg, rs2076530 (A) pos	82 *versus* 70	0.008	2.0 (1.20–3.34)				87 *versus* 70	0.002	2.8 (1.42–5.33)			
*DPB1*04:02* neg, *DRB1*01:01* neg	72 *versus* 52	<0.001	2.3 (1.49–3.66)	69 *versus* 52	0.012	2.0 (1.16–3.49)	75 *versus* 52	<0.001	2.7 (1.55–4.70)			
*DRB1*01:01* neg, *DPB1*04:02* neg, rs2076530 (A) pos	68 *versus* 49	<0.001	2.2 (1.43–3.48)				74 *versus* 49	<0.001	2.9 (1.68–5.07)			

Sarcoidosis n=187, resolved sarcoidosis n=89, persistent sarcoidosis n=98, controls n=150. One patient with resolved sarcoidosis failed the rs2076530 genotyping and was excluded from the analyses. A value of $p<0.05$ is considered statistically significant. The p-values are uncorrected. The positivity (pos) of a carrier of a specific variant (or variants) was considered when the subject had one or two copies of the variant. Neg: negativity or absence of the variant. [#]: significant association with sarcoidosis in our previous study: *HLA-DRB1*01:01*, **03:01* and **15:01* [8]. [¶]: the logistic regression model (stepwise forward) was used for studying the relationship between the significantly associated markers and the disease, to estimate the effects of individual markers. [+]: Chi-squared analysis.

Altogether, 19 different *HLA-DPB1* alleles were observed, from which alleles **04:01*, **04:02*, **02:01*, **03:01*, **01:01* and **05:01* were considered common (phenotype frequency >5%). We could not confirm the association between sarcoidosis and the CBD-related marker *HLA-DPB1*02:01* (or Glu69). However, we found a significant decrease in the *HLA-DPB1*04:02* phenotype frequency among sarcoidosis patients when compared with the controls (22% *versus* 37%; p=0.003, OR 0.48, 95% CI 0.30–0.79). *HLA-DPB1*04:02* appears to protect especially against the persistent, chronic type of sarcoidosis (19% *versus* 37%; p=0.004, OR 0.42, 95% CI 0.29–0.76).

Our study replicates the *BTNL2* splice site polymorphism (A variant of rs2076530) showing association with an increased risk for persistent sarcoidosis when compared with the controls (carrier frequencies 92.9% *versus* 84.0%; p=0.039, OR 2.48, 95% CI 1.02–5.99). The frequency distribution of rs2076530 did not differ significantly between subgroups or between the resolved group and the controls. *BTNL2* is a member of the immunoglobulin gene superfamily and has an important role in the interaction between B- and T-lymphocytes and in affecting T-cell proliferation [5]. The truncation of BTNL2 protein may affect normal T-cell regulation and antigen response [5, 6]. However, the *BTNL2* splice site polymorphism is rather common in the healthy population and typically inherited within conserved MHC haplotypes. Thus, it is plausible that other causal variants exist and should be taken into account.

Previously, *HLA-DRB1*04* has been proposed to associate with Heerfordt's syndrome, uveitis and ocular sarcoidosis [2, 9, 10], but no association with *HLA-DRB1*04:01* and the disease course of sarcoidosis has been published. In our study the frequency of haplotype *DRB1*04:01-DPB1*04:01* was increased in resolved sarcoidosis when compared with the controls (16.9% *versus* 7.3%; p=0.02, OR 2.6, 95% CI 1.12–5.86) or persistent sarcoidosis (16.9% *versus* 6.1%; p=0.02, OR 3.1, 95% CI 1.15–8.41). The linkage disequilibrium between *HLA-DRB1*04:01* and *HLA-DPB1*04:01* alleles was stronger in the resolved group than in the controls or in the persistent group (D'=0.72, D'=0.22, no linkage disequilibrium, respectively), indicating that the haplotype is enriched in the resolved group. Furthermore, studies show that *DRB1*04:01-DPB1*04:01* association is independent of the well-known predisposing genes *HLA-DRB1*03:01* and *HLA-DRB1*15:01* (table 1) [8].

The haplotype analysis showed that certain *DRB1-DPB1* haplotypes can be described as either predisposing (*rs2076530(A)-DRB1*03:01-DPB1*01:01*) or protective (*rs2076530(G)-DRB1*01:01-DPB1*04:02*), corroborating the previous allele associations. Conversely, the same rs2076530 polymorphism can be found both in susceptibility (*e.g. rs2076530(G)-DRB1*04:01-DPB1*04:01*) and in protective haplotypes (*e.g. rs2076530(G)-DRB1*01:01-DPB1*04:02*).

Distinct linkage disequilibrium between markers hampers the MHC studies. Many studies have discussed whether the rs2076530 association is secondary to MHC class II association [6]. *HLA-DRB1*01:01* is in a strong linkage disequilibrium (D'>0.80) with rs2076530 but forms different haplotypes with *HLA-DPB1* alleles. In this regard, we investigated further the carriage of *BTNL2* rs2076530(A), *HLA-DRB1*01:01*, *HLA-DPB1*04:02* and their combination (table 1). The presence of *BTNL2* rs2076530(A) and absence of at least one protective class II molecule (*HLA-DPB1*04:02* or *HLA-DRB1*01:01*, or both) had the highest predisposition ratios for persistent sarcoidosis (table 1). A similar trend was shown when the whole sarcoidosis group was analysed. Our results indicate that both MHC class II (antigen presentation) and *BTNL2* rs2076530(A) (T-cell regulation) are necessary risk factors for susceptibility to develop persistent sarcoidosis (table 1).

Our study has some limitations. We increased the statistical power by using a well phenotyped patient group and haplotypes rather than alleles. As the controls were collected from a healthy adult population, we cannot exclude the possibility that the control group may include subjects who have gone on to develop sarcoidosis. We are also aware that environmental factors are probably needed for predisposition to sarcoidosis, but unfortunately we do not have specific exposure data available (*e.g.* beryllium exposure). The lack of exposure data would have been more of a problem if we had found the CBD marker Glu69 in our sarcoidosis patients, which we did not.

In conclusion, our study shows that resolved and persistent sarcoidosis have different combinations of disease-related MHC markers (*HLA-DRB1*, *-DPB1* and *BTNL2* rs2076530). Therefore, we highlight the importance of accurate phenotypic categorisation of sarcoidosis patients and underline the necessity of studying wider regions of the MHC in order to investigate independent risk factors. The prognostic value of the MHC markers should be evaluated in larger cohorts and in different populations.

@ERSpublications
A Finnish study shows that resolved and persistent sarcoidosis have different combinations of disease-related MHC markers http://ow.ly/l5ZaU

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Received: Feb 26 2013 | Accepted after revision: Feb 28 2013

Support statement: This study was supported by the Nummela Foundation for Medical Research, Hengityssairauksien tutkimussäätiö, the Helsinki Biomedical Graduate Programme (HBGP), and the League of European Research Universities (LERU).

Conflict of Interest: Disclosures can be found alongside the online version of this article at www.erj.ersjournals.com

Acknowledgements: The authors thank the patients for their participation and the clinicians for their time and efforts to make this study possible. We also thank L. Saraste (Transplantation Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland) for reviewing the English language and M. Veini, L. Snellman, K. Roine, E. Lahtela (all at the Transplantation Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland) and M. Kaunisto (FIMM, Institute for Molecular Medicine Finland, Biomedicum, University of Helsinki, Helsinki) for their contribution to gene analyses.

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Eur Respir J 2013; 42: 550–553 | DOI: 10.1183/09031936.00035213 | Copyright ©ERS 2013

Reversibility of the monocrotaline pulmonary hypertension rat model

To the Editor:

Pulmonary hypertension (PH) is a disease characterised by progressive remodelling of the pulmonary vasculature eventually leading to right heart failure. Various animal models have been used to mimic the disease, involving pigs, dogs, rats and mice [1]. The most commonly used model is the monocrotaline (MCT) rat model. In this model MCT is injected subcutaneously and becomes metabolically activated, as a pyrrolizidine alkaloid, by hepatic cytochrome P450 3A [2, 3]. The active MCT pyrrole is pneumotoxic and damages the pulmonary artery endothelial cells (PAECs), which leads to a disturbed barrier function [4]. Other features of MCT-induced pulmonary vascular remodelling are arterial medial hyperplasia of axial arteries, interstitial oedema, adventitial inflammation, haemorrhage and, eventually, fibrosis [1, 2, 5, 6]. As a result, pulmonary vascular resistance (PVR) increases and the right ventricle compensates by hypertrophy and eventually fails [7, 8].