

HLA associations and Löfgren's syndrome

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Patients with sarcoidosis can be subgrouped according to organ engagement and clinical manifestations. One such subgroup is Löfgren's syndrome (LS), constituting a distinct group of sarcoidosis patients with typical clinical manifestations, separate genetic associations and an immune response that seems to differ from that of non-LS patients. In particular, LS patients have strong associations with *HLA-DRB1* alleles, and the well-known association with *HLA-DRB1*03* is particularly striking. This particular *HLA-DRB1* allele is also a very strong marker within that particular group of patients for a prognostically favorable disease course. This article will mainly discuss genetic associations with LS, and the possible implications of such associations.

KEYWORDS: HLA • Löfgren's syndrome • phenotype • sarcoidosis • T cells

Background

A genetic influence on sarcoidosis is implicated by findings of an increased risk for disease among patients' family members, as well as increased risk for monozygotic twin siblings, and distinct disease manifestations in different ethnic groups. Although sarcoidosis is a genetically complex disease, in that there are many genes contributing to the increased risk for disease, the strongest associations are found within the HLA region on chromosome 6, which besides the HLA-class I and class II genes includes a large number of genes important for an immune response [1–7]. Such associations indicate the presence of specific antigens that are preferentially presented by certain HLA alleles, thereby inducing a sarcoidosis immune response eventually leading to granulomatous inflammation in these patients. The typical presence of large T-cell populations with restricted sets of T-cell antigen receptors (TCR) in the lungs of sarcoidosis patients is well in line with such a hypothesis [8–12].

Löfgren's syndrome: definition

Löfgren's syndrome (LS) is an acute form of sarcoidosis, usually with fever, characterized on chest radiography by bilateral hilar lymphadenopathy (BHL; in some cases with parenchymal infiltrates), erythema nodosum (EN) and/or bilateral ankle arthritis or distinct periarticular inflammation [13]. Our clinical impression was that patients with acute onset and BHL on chest radiography, with bilateral ankle

arthritis but without EN, sometimes referred to as 'sarcoid arthritis' [14,15], should be considered to be a variant of LS [16,17]. We therefore evaluated 150 Scandinavian patients with an acute onset of sarcoidosis and BHL; 87 of these patients had EN, while 63 cases were without EN but had marked periarticular inflammation or arthritis of the ankles [16]. The majority of patients with EN (80%) had bilateral ankle arthritis concomitantly. There was no difference in age between patients with or without EN, but there was a significant dominance of women among patients with EN; 67% women compared with 27% among patients without EN. The female dominance among patients with EN was also persistent in patients above 45 years of age (84% women among patients with EN) [16].

There was a majority of never-smokers in both groups, BHL with pulmonary infiltrates (stage 2) were found in 23% of the patients in both groups and pulmonary function tests were identical. Bronchoalveolar lavage fluid (BAL) cell differential counts and CD4:CD8 ratios were close to identical in both groups, as well as the seasonal distribution of disease onset, with a preference for the first months of the year. Importantly, the disease outcome was similar in both groups, as more than 80% of all patients in both groups recovered within 2 years. Finally, in both groups there was a close to identical linkage to a certain HLA allele (*HLA-DRB1*0301/DQB1*0201*), with approximately 70% of the patients in both groups expressing this allele. In

conclusion, we found LS patients with or without EN to be essentially identical from a clinical, immunological, inflammatory and genetic point of view. Thus, LS should include patients with an acute onset of the disease with BHL and bilateral periarticular inflammation of the ankles, even when they do not have EN [18].

Incidence of LS

LS occurs rather frequently in Scandinavia, where approximately a third of all sarcoidosis patients have LS [19]. In the USA, only five of a total of 736 patients (0.7%) were classified as having LS in the multicenter ACCESS study [20], and in a recent study by Sato *et al.*, only three out of 340 (0.9%) UK sarcoidosis patients were classified as having LS [21]. In the same study, none of the 163 patients in Japan were found to have LS, although such case reports have been previously described [22]. In addition, the study by Sato *et al.* classified as many as 47 out of 139 (33.8%) of Dutch patients to have LS [21]. In a retrospective study from one medical center in Istanbul, Turkey, 98 out of 514 sarcoidosis patients (19.1%) were diagnosed with LS [23]. In Spain, LS has been reported to be most common, constituting approximately half of all sarcoidosis patients [24]. Some of these differences might be explained by a bias in patient selections, for example, through differences in the patient referral systems and/or inclusion criteria.

Genetic differences between distinct ethnic groups may also explain variations in the frequency of LS in various populations. As for other sarcoidosis patients, LS is believed to develop in genetically predisposed individuals that are exposed to unknown antigens. A genetic influence on sarcoidosis is implicated by the tendency of familial clustering of the disease, and by the finding that family members of sarcoidosis patients have an increased risk for the disease; one large US-based study estimated a fivefold increased risk [25–27]. Monozygotic twins have been estimated to have an 80-fold increased risk for developing sarcoidosis if one of the twins has sarcoidosis, compared with only a sevenfold increased risk among dizygotic twins [28]. Interestingly, in spite of the increased risk for family members, there seems to be only weak similarities of the affected siblings disease phenotypes [29].

An additional argument for genetic factors being important for developing sarcoidosis is the fact that clinical manifestations and the disease course differ between distinct ethnic groups [30]. For example, African-Americans are affected more frequent than white Americans, and they develop a severe disease more frequently [5]. In Japan, compared with Finland, while ocular sarcoidosis is significantly more frequent, EN is less common [31,32].

HLA associations with LS

Previous studies indicate the HLA region on the short arm of chromosome 6 to be the most important for the genetic contribution to sarcoidosis [7,33]. Distinct variations in this region not only confers an increased risk for disease (FIGURE 1), but also associates with clinical features and the disease course [34]. Strong associations between distinct HLA class I (*HLA-B8*) and class II (*HLA-DRB1*03*) alleles and acute onset, EN or good prognosis were described some 30 years ago [35–37]. Subsequent studies, using more specific tools for HLA analyses, confirmed and expanded the findings of the strong links between *HLA-DRB1*0301*, LS and good prognosis [38–43]. With the strong association between *DRB1*03* and LS in mind, in Japan, where *DRB1*03* is virtually missing, LS is accordingly very rare [21,22]. By contrast, in countries like Sweden, The Netherlands and Spain, where *HLA-DRB1*03* is rather common, the LS frequency is also high [19,21,24]. Although LS, as well as *HLA-DRB1*03*, is rather uncommon in individuals with an African ancestry [20], *HLA-DRB1*03*-associated LS may appear in African-born individuals. We previously described an African-born *DRB1*03*-positive individual who was at an early age adopted by Swedish parents, grew up in Sweden, and as an adult developed LS. Interestingly, she also had the previously described *DRB1*03*-associated immune response characterized by lung-accumulated T cells expressing the TCR *AV2S3* gene segment [44].

Distinct parts of HLA molecules are important for antigen binding

Chronic beryllium disease (CBD), which may well have been recognized as sarcoidosis before its specific pathogenesis was revealed, is characterized by strong associations with *HLA-DPB1* alleles with a glutamic acid at the 69th position (Glu69) of the DPB β -chain. It has been known that beryllium presentation mainly occurs through Glu69-containing HLA-DP molecules, resulting in lung and blood T-cell responses in sensitized individuals [45–47]. Through crystallization of *HLA-DPB1*0201*, that is, the most prevalent DP allele with Glu69, Falta *et al.* recently found a structural explanation for this genetic linkage [48]. They showed that Glu69 together with Glu68 and Glu26 constituted a distinct negatively charged pocket, which was unusually exposed and suggested to directly bind beryllium. In sensitized individuals, HLA-DP presentation of beryllium can in this manner activate

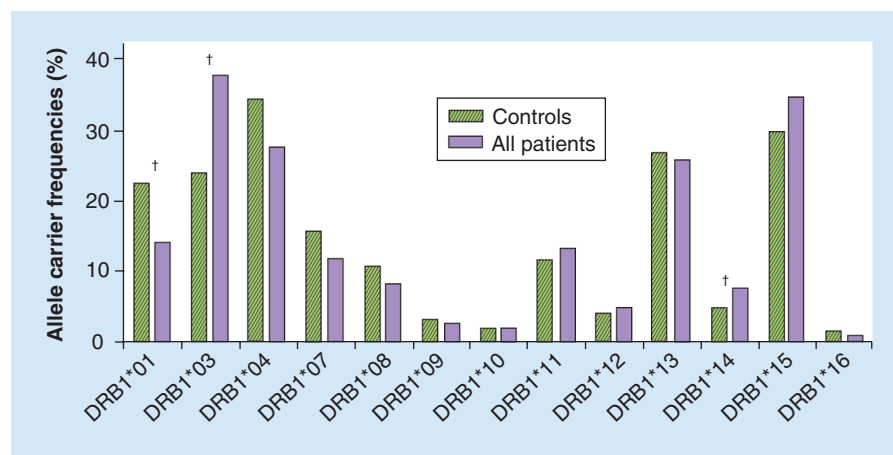


Figure 1. Allele carrier frequencies in healthy controls (controls) and overall sarcoidosis (all patients).

†Statistically significant differences.

T cells and subsequently generate inflammation and granuloma formation, and thus explain the strong genetic linkage between Glu69-containing *HLA-DP* alleles and CBD [48,49].

In sarcoidosis, the protective properties of *HLA-DRB1*01* and *DRB1*04* have been suggested to be caused by the presence of certain hydrophobic amino acids at position 11 of pocket 6 of the DRB1 molecule [50], which would influence antigen peptide binding and eventually drive the immune response to more effectively eradicate potentially sarcoidosis-generating antigens. Furthermore, pocket 4 of the DRB1 molecule has been suggested to be important for associations with sarcoidosis. An independent association between Arg74 in pocket 4 and prognostically favorable disease was shown by Voorter *et al.* [51]. In our own studies, we found the same pocket 4 of DRB1 to strongly associate with accumulations of AV2S3 TCR-positive T cells, indicating specific antigen peptide presentation in the lungs of these patients [52]. In a recent study by Sato *et al.*, support is presented for the importance of DRB1 pocket 4 for associations with the clinical sarcoidosis phenotype [21]. Saltini *et al.* used bioinformatic tools to describe that LS-associated *DRB1* alleles could recognize significantly higher numbers of bacterial antigen epitopes, and in particular, such epitopes derived from mycobacteria [53]. In all, *DRB1* allele associations may be due to shared epitopes in regions of importance for antigen-peptide binding (so-called pockets).

Linkage disequilibrium in the HLA region

Although the above findings are in line with the hypothesis that binding and presentation of specific antigen peptides by DRB1 molecules is important for sarcoidosis pathogenesis, there are other plausible explanations for the strong associations between the HLA region and sarcoidosis. This is because of the linkage disequilibrium (LD) phenomena, by which distinct gene variants are inherited together more frequently than expected. Thus, the described associations might merely reflect other, true associations with closely located genes that may also be of importance for the immune response. *HLA-DRB1*03* is, for example, part of a highly conserved haplotype (i.e., closely linked genetic markers on a chromosome that are transmitted together), called the 8.1 ancestral haplotype, which is associated with a number of autoimmune disorders, such as myasthenia gravis and systemic lupus erythematosus [54]. This haplotype includes among others *HLA-A1*, *-B8* and *DQB1*0201* alleles, as well as a *C4*-null allele and the *TNF- α -308 A* allele, which is characterized by a high *TNF- α* production. The absence of *C4* may disturb the clearance of immune complexes and promote inflammation [55]. In fact, *HLA-DRB1*0301* and *DQB1*0201* are so strongly linked together in Caucasian populations that it may be difficult to sort out which of the two is primarily linked to a favorable disease course [54,56]. Interestingly, among African-Americans, *DQB1* rather than *DRB1* alleles have been suggested to be more important for sarcoidosis development [57].

The pronounced LD phenomena in the HLA region is thus complicating interpretations of disease associations with specific gene variants and any associations should be interpreted with caution. One gene variant in the HLA class II region that was more recently was found to associate with sarcoidosis is the *BTNL2 G16071A*

gene variant (rs2076530), also termed the *A* allele. *BTNL2* encodes a receptor related to CD80 and CD86 costimulatory receptors, and may possibly have inhibitory effects on T cells [58]. The *BTNL2 A* allele has been suggested to cause a functional change that disrupts the membrane localization, and thus function of the protein [58]. Subsequent studies have confirmed the association with sarcoidosis, and Li *et al.* found the association to be true only for with the chronic form of sarcoidosis [59]. Also Spagnolo *et al.* identified certain *BTNL2* variants to associate with chronic sarcoidosis, and suggested that LD with certain *HLA-DRB1* alleles could explain such associations [60]. Recently, Wijnen *et al.* analyzed 632 sarcoidosis patients and subgrouped them into those with persistent (n = 304) or nonpersistent (n = 328), and described a significant association between persistent disease and the *BTNL2 A* allele, which in turn was shown to be strongly linked to *HLA-DRB1*15*. They concluded that the increased risk for sarcoidosis as described for *BTNL2A* is caused by its strong linkage to *DRB1*15* [61].

HLA distribution differs in LS versus non-LS patients

In a recent study, we found the HLA allele carrier frequencies to differ significantly in LS-patients compared with non-LS patients [19]. In particular, *DRB1*01*, *DRB1*03* and *DRB1*14* associations differed between LS and non-LS patients (FIGURE 2). Thus, while *DRB1*01* strongly protected against disease in non-LS patients, there was no influence in LS, and while *DRB1*03* was a very strong risk factor for LS, it was protecting against non-LS. Also, *DRB1*14* associated with non-LS but was not linked to LS. In some cases, other gene variants have also been found to associate with LS only. For instance, a certain *CCR2* haplotype 2 (a chemokine receptor for monocyte chemoattractant proteins of importance for recruitment of monocytes, T cells and NK cells) was found to associate with LS patients in both Sweden and Spain, but not with non-LS patients [62]. Using multivariate analysis, this *CCR2* association was shown to be independent of *DRB1*03*, thus constituting a separate genetic risk factor for LS [62]. Another recent example is the MHC class II transactivator (*MHC2TA*), regulating the expression of HLA class II molecules. Analyzing 728 sarcoidosis patients and 873 controls, we found a significant association with LS and the rs3087456 single nucleotide polymorphisms, which was not detected in non-Löfgren's patients, and moreover this association seemed to be independent of *HLA-DRB1*03* [19].

On the other hand, there are also similarities between the two subgroups LS and non-LS sarcoidosis patients, in particular regarding *HLA-DRB1* allele associations with disease chronicity. In both subgroups, *DRB1*03* associated with a prognostically favorable disease course, albeit more so in LS patients, while *DRB1*07*, *DRB1*14* and *DRB1*15* in both patient groups tended to associate with a more chronic form of the disease [19]. In general, while the *DRB1*03* association with a favorable disease course has been reproduced in many populations, this is not the case to the same degree for associations between *HLA-DRB1* alleles and chronic disease. Perhaps a chronic disease course is more difficult to define, studies have included some patient and/or control biased selections, or, the influence on the sarcoid inflammation and the disease course by *DRB1*15*, for example, may not be that strong.

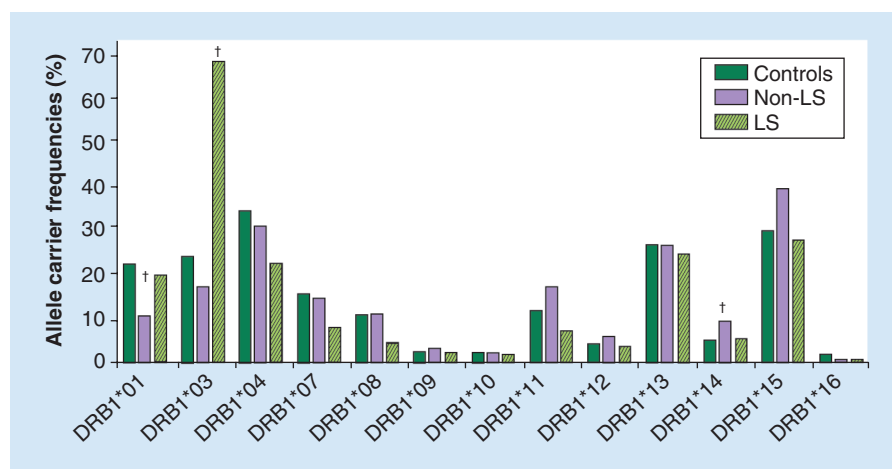


Figure 2. Allele carrier frequencies in healthy controls, non-Löfgren's syndrome sarcoidosis and Löfgren's syndrome sarcoidosis.

*Statistically significant differences compared with healthy controls.
LS: Löfgren's syndrome.

In future studies of gene associations in sarcoidosis, one may thus carefully clinically phenotype and separately analyze LS patients, in order to improve the chance to reveal true genetic associations.

Another distinct sarcoidosis syndrome is Heerfordt's syndrome (HS), that is, patients with fever and the characteristic combination of uveitis, parotid or salivary gland enlargement and cranial nerve palsy. In a recent study we compared the distribution of *HLA-DRB1* alleles in 1000 sarcoidosis patients with 2000 age- and sex-matched healthy controls, and in particular studied patients with HS [63]. However, because the complete HS syndrome was very unusual, we included patients with any of the symptoms related to HS, and there found a significant overrepresentation of the *HLA-DRB1*04* allele. Among patients with any of the HS-associated symptoms ($n = 83$ out of 1000), more than half were *DRB1*04*-positive, as compared with a third of healthy controls and to only a quarter of the entire sarcoidosis cohort (*DRB1*04* is negatively associated with, or protecting against, overall sarcoidosis). Thus, another subgroup of patients seems to exist with a distinct set of clinical manifestations, which is characterized by strong associations with a particular *HLA-DRB1** allele (*DRB1*04*). Interestingly, Sato *et al.* recently described *DRB1*04-DQB1*0301* to strongly associate with uveitis (the most common HS-associated symptom) in Japan, as well as in UK patients [21]. One clinical implication may be to monitor any *DRB1*04*^{pos} sarcoidosis patient more closely with regard to HS-associated symptoms, and in particular for uveitis [63].

HLA-DRB1*03 associates with the disease course in LS patients

As previous studies had indicated an association between *DR3* and good prognosis [35,36,38,39,41,42,56], in a recent report we analyzed and compared *DR3*^{pos} and *DR3*^{neg} LS patients, and found dramatic differences. Thus, in almost every case, *DRB1*03*^{pos} LS patients recovered within 2 years, while only half of the *DRB1*03*^{neg} LS patients ran the same favorable disease course [64].

Among *DRB1*03*^{neg} LS patients, especially *DRB1*04* and *DRB1*15* seemed to associate with a prolonged disease course, while *DR1* and *DR13* instead correlated with a short disease course. The majority of *DRB1*03*^{neg} LS patients that were treated with oral steroids had a prolonged disease course; 80% had signs of disease for more than 2 years, while such treatment did not influence the very good prognosis of *DRB1*03*^{pos} LS patients. Studying the time of the year for disease onset, we found, as others have also previously described [65–67], a clustering around the spring months, although we could detect an additional peak in January [64]. Interestingly, this clustering was not seen in *DRB1*03*^{neg} LS patients, where there was no distinct preference for disease onset over the year. The clustering in time for disease onset only for

*DRB1*03*^{pos} LS patients may indicate a specific gene–environment interaction leading to disease only in patients with a ‘permissive’ HLA allele, that is, in this case *DRB1*03*, upon exposure for certain antigens.

The difference in the disease course for *DRB1*03*^{pos} and *DRB1*03*^{neg} LS patients is not quite understood, but it is clearly established that *DRB1*03*^{pos} patients have large populations of T cells with a distinct antigen specificity accumulating in their lungs (*TCRAV2S3*^{pos} T cells), and that such T-cell expansions correlate well with disease activity. One explanation would therefore be that such *AV2S3*^{pos} T cells, found compartmentalized in the lungs of *DRB1*03*^{pos} patients with active sarcoidosis, are capable of efficiently eradicating sarcoidosis-associated antigens and through this associate with good prognosis. Interestingly, Amicosante *et al.* hypothesized that since 50% of the *DRB1*03*^{neg} patients also have a favorable disease course, such patients should have other *DRB1* alleles capable of inducing an immune response leading to disease resolution. Using an *in silico* model to measure affinity for bacterial antigen epitopes, *DRB1* alleles associated with LS were found to recognize significantly higher numbers of bacterial antigen epitopes than did other *DRB1* alleles, but combinations of non-*DRB1*03* alleles could also generate high binding affinities [68]. Applying an *in silico* model on candidate antigens may reveal relevant *DRB1* amino acid sequences of importance for antigen peptide binding and that may associate with the disease course in sarcoidosis, thus helping to identify individuals at risk for developing a chronic disease [53].

One may also analyze and compare in more detail *DRB1*03*^{neg} LS patients who recover with those developing a prolonged disease course, possibly to find any differences that could explain the sharp difference in disease outcome. Whatever the mechanism may be, we now routinely *HLA-DRB1*-type all our patients evaluated for sarcoidosis, and we use the result to help predict the clinical course of the disease, with implications for monitoring and treating patients.

Implications for the immune response

Associations with *HLA-DRB1* alleles suggest that presentation of distinct antigen peptides are of importance in sarcoidosis. This is in line with the typical finding of lung-compartmentalized and highly activated CD4⁺ T cells, resulting in the characteristically elevated BAL fluid CD4:CD8 ratio, a feature that is of help in the clinical evaluation of the patient. In fact, the interaction between APC and CD4⁺ T cells is regarded as crucial for the subsequent inflammatory process, eventually giving rise to granuloma formation and, in some cases, chronic inflammation and fibrosis. A number of studies have also shown that the compartmentalized CD4⁺ T cells express a limited set of TCRs, that is, their antigen specificity is restricted [8,69,70]. In particular, the finding of a very strict correlation between *HLA-DRB1*03* and the lung accumulation of large CD4⁺ T-cell populations expressing a distinct TCR α chain V gene segment (AV2S3) strongly suggests the presence of specific antigens in sarcoidosis [9,10,71]. Further support of the hypothesis of specific antigen presentation in sarcoidosis is the finding that Swedish sarcoidosis patients expressing *HLA-DRB3*01*, which has similar antigen peptide-binding capacities as *DRB1*0301*, also have lung accumulated *TCR AV2S3*-positive T cells [72].

Sarcoidosis is suggested to be an autoimmune disorder, for example, initiated through crossreactivity between microbial and human proteins [73]. The strong associations with HLA alleles further implicates autoimmunity, in which APCs are capable of presenting self antigens in such a way that T cells are activated and subsequently initiate autoimmune reactions. Tolerance might be broken if self-peptides are modified, for example, citrullinated as in the case of rheumatoid arthritis [74], or if self-peptides that are normally sequestered for the immune system are presented by HLA molecules. In sarcoidosis, using a new technique to determine exactly what antigenic peptides are presented by the HLA molecules on BAL cells, several self proteins were identified [75]. At least one of these peptides (i.e., derived from vimentin) was in addition capable of stimulating T cells of sarcoidosis patients but not healthy controls, indicating autoimmunity as part of the pathogenic processes in sarcoidosis [76].

HLA class I molecules normally present endogenously derived antigens for CD8⁺ T cells, as in viral infections, and class I alleles are in LD with the HLA class II region. It may thus be difficult to evaluate any separate influence of HLA class I alleles on the risk for sarcoidosis, or on the disease course. However, one study suggested *HLA-B*07* to be an independent risk factor for sarcoidosis, and that the combination of *HLA-A*03* and *-B*07* significantly increased the risk for chronic disease in *HLA-DRB1*15^{pos}* patients [41]. Although the role for CD8^{pos} T cells in sarcoidosis is far from understood, they are an important source of key cytokines, such as IFN- γ [77], and may have functions in the pathogenesis of sarcoidosis that are not yet recognized.

Expert commentary

Although sarcoidosis is considered to be a genetically complex disease, the strongest associations by far are found with the HLA region, including genes coding for HLA molecules that have a pivotal role in the immune system by presenting antigens for T lymphocytes.

A number of distinct HLA molecules have been found to associate with the risk for sarcoidosis, as well as with the course of the disease.

The data presented in this article show the importance of subgrouping patients in order to reveal genetic associations. For instance, when analyzing patients with LS separately, new gene associations are revealed. The strong link between clinical manifestations and distinct HLA alleles, as well as between particular HLA alleles and the disease course, opens the possibility of using such associations in the clinic, in order to improve the clinical management and enable individualized treatment.

Sarcoidosis seems to be particularly suitable for this type of subgrouping of patients, most typically manifested by LS patients, which perhaps constitutes a separate disease entity, but also to some degree by patients with HS-associated symptoms. It has been suggested that the sarcoidosis diagnosis covers a collection of specific disease entities; obviously with parts of the pathogenesis in common, such as granuloma formation, but possibly also with distinctions related to etiology, clinical manifestations and disease course. In such a perspective, CBD might be regarded as a variant of sarcoidosis in which the etiology has been more or less clarified (and is therefore no longer classified as sarcoidosis). Furthermore, approximately half of the sarcoidosis patients have specific immune responses against mKatG of *Mycobacteria*, indicating a distinct etiology for another subgroup of patients [78,79]. Thus, HLA-typing sarcoidosis patients will have clinical implications by improving the management of the patient; it may help to identify organ engagements that may not be obvious in a routine evaluation (such as HS) and improve our ability to predict the disease course (in LS and, to some extent, all patients).

Five-year view

In the future, it is reasonable to anticipate further subgrouping of patients with sarcoidosis, for example, into patients with specific organ manifestations, which will reveal new genetic associations that may not only be of clinical relevance, but may also help to understand the pathogenesis of this intriguing disorder.

Moreover, we may in the coming years learn more about and even unravel the etiology for subgroups of patients. For example, before it was understood that beryllium was the triggering factor for developing CBD, those patients were probably classified as having sarcoidosis. In the future, we may learn that in susceptible individuals, sarcoidosis might develop as a consequence of exposure for *Mycobacteria*. Therefore, gene–environmental interactions will be a new and important area to explore.

Financial & competing interests disclosure

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Key issues

- Although many genes contribute to sarcoidosis, the strongest associations are found within the HLA region on chromosome 6, which in addition to the HLA-class I and class II genes, includes a large number of genes important for an immune response.
- In some cases, such HLA allele associations are of clinical importance, improving prognostic accuracy and are of relevance for how to monitor and possibly treat the patients.
- Such gene associations suggest the presence of specific antigens that are preferentially presented by certain HLA alleles.
- The accumulation of CD4^{pos} T helper cells to the lungs, with restricted sets of T-cell antigen receptors, is also compatible with the hypothesis of specific antigens being presented by HLA molecules.
- HLA molecules contain specific 'pockets' that are of importance for binding and presenting specific antigen peptides, and the composition (i.e., amino acids) of such pockets has been associated with sarcoidosis.
- Erythema nodosum is not strictly required to diagnose Löfgren's syndrome, since patients without erythema nodosum but instead with marked periarticular inflammation or arthritis of the ankles (usually men) have an identical disease as evaluated clinically, immunologically and genetically.
- Among patients with Löfgren's syndrome, the presence (or not) of *HLA-DRB1*03* dictates the prognosis, and HLA-typing is therefore a valuable tool to help predict the disease course.
- Sarcoidosis patients who are *HLA-DRB1*04* should be closely monitored for symptoms related to Heerfordt's syndrome (uveitis, parotid or salivary gland enlargement and cranial nerve palsy).
- It is crucial to subgroup and phenotype patients clinically in order to reveal true gene associations.

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