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Original Research

HLA-DRB1 alleles associate with hypercalcemia in sarcoidosis

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ABSTRACT

Background: The mechanisms behind and which patients are at risk of developing sarcoidosis associated hypercalcemia (SAHC) have not been addressed. Different human leukocyte antigen (HLA) alleles associate with disease phenotypes in sarcoidosis. Insights into associations between HLA alleles, clinical phenotype and calcium levels may provide clues to mechanisms behind SAHC and help monitoring patients at risk for SAHC.
Aims and objectives: To identify any HLA-association with SAHC, and to phenotypically characterize this patient group.
Methods: 66 patients with SAHC (s-Ca^{2+}>1.33 mmol/L) and 150 normocalcemic patients as controls were identified in a cohort of sarcoidosis patients. Data on *HLA-DRB1* alleles, sex, angiotensin-converting enzyme (ACE), creatinine, extrapulmonary manifestations (EPM), age at sarcoidosis diagnosis, and how long after diagnosis SAHC emerged, were retrieved.
*Results: HLA-DRB1*04* was more common in patients with SAHC and the proportion of patients with *HLA-DRB1*04* increased the more pronounced hypercalcemia. In patients with s-Ca^{2+}>1.4 mmol/L, 20 out of 30 carried the *HLA-DRB1*04* allele (67%, $p < 0.01$). Patients with SAHC more often disclosed renal insufficiency, elevated ACE, EPM, and a non-resolving disease than controls. The mean duration between sarcoidosis diagnosis and detection of SAHC was 1.39 years.
Conclusions: SAHC is associated with a more severe disease phenotype, particularly patients carrying the *HLA-DRB1*04* allele are at higher risk for SAHC. HLA-assessment in the clinic can be a way to identify these patients. The results provide a basis for future studies on the connection between *HLA-DRB1*04* and SAHC mechanisms.

1. Background

Sarcoidosis is an inflammatory disease of unknown etiology, associated with non-necrotizing granulomas. In 90% of cases, the lungs and/or intrathoracic lymph nodes are affected, but the disease can occur in almost any organ [1,2]. The onset can be acute and often self-limiting, which is more commonly seen in the clinical phenotype Löfgren's syndrome (LS), or with a slower debut that more often results in a non-resolving disease, more commonly seen in non-Löfgren's syndrome (non-LS). A non-resolving disease can lead to organ function impairments and, sometimes, failure [1]. Genetic factors influence the risk of sarcoidosis, and different *HLA-DRB1* alleles are associated with the disease and target-organ phenotypes [3]. For instance, *HLA-DRB1*04* is associated with extrapulmonary manifestations (EPM), chiefly ocular sarcoidosis [4–6], *HLA-DRB1*03* with Löfgren's syndrome and resolving disease, and *HLA-DRB1*14* and *HLA-DRB1*15* are associated with a non-resolving disease course [7,8].

Since the 1930's, it has been known that patients with sarcoidosis can develop SAHC [9], which can affect the patients' general condition and damage internal organs, e.g., kidneys leading to renal insufficiency.

The mechanisms behind and which patients may have an increased risk of developing SAHC are not fully understood. Studies have reported an increased risk for patients of white European ancestry, male sex, and over 40 years of age [10,11], while another study could not find any

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connection to race, sex or age [12]. A study from the US showed that *HLA-DRB1*1101* was more frequent in white patients with SAHC, but not in patients of black African ancestry [5].

Active vitamin D, 1,25-(OH)2 vitamin D3, increases the uptake of calcium from the intestines as well as enhances bone resorption and is one cause of SAHC. It is formed by 1-alpha hydroxylase that activates 25-(OH) vitamin D3 to the active form 1,25-(OH)2 vitamin D3. This process is usually conducted in the kidney, but studies have shown that monocytes also have 1-alpha hydroxylase and the ability to activate vitamin D3 [13–15]. It is further showed that 1-alpha hydroxylase present in pulmonary alveolar macrophages (PAM) are activated by interferon-gamma [16] and do not have the same feedback loop as the hydroxylation process that is conducted in the kidney or peripheral blood monocytes (PBM) [17]. This has been suggested to be one of the mechanisms behind SAHC [18–20]. Kavathia et al. showed in 2010 that increased levels of 1,25-(OH)2 vitamin D3 are associated with the prolonged need for therapy and increased odds of a non-resolving phenotype. It is also discussed as an immunomodulating hormone [21].

However, serum 1,25-(OH)2 vitamin D3 can also be normal [12,22] in patients with SAHC, and one study found no correlation between serum calcium and 1,25-(OH)2 vitamin D3 [23].

Another suggested mechanism is the production of parathyroid hormone-related peptide (PTHrp) in the granulomas causing SAHC through enhanced bone resorption and tubular calcium reabsorption [24,25].

Insight into the influence of *HLA-DRB1* alleles and patient phenotype may provide mechanistic clues and help to identify patients at risk of developing SAHC. Therefore, this study was designed to assess the association of *HLA-DRB1* alleles and clinical characteristics with SAHC, using a cohort with sarcoidosis patients.

2. Materials and methods

2.1. Study subjects

A cohort of 1229 patients with sarcoidosis from our local registry at Karolinska University Hospital, Stockholm Sweden, containing clinical and genotype data collected between 1987 and 2018, was retrospectively searched and combined with medical records to find patients with SAHC. Using the criteria outlined by the World Association of Sarcoidosis and other Granulomatous Disorders (WASOG), subjects were diagnosed through typical clinical and radiographic manifestations, findings at bronchoscopy including elevated CD4/CD8 cell ratio and positive biopsies [1]. All included patients had signed a written consent form, and approval was granted from the regional ethical review board.

Included subjects were checked to not be on vitamin D or calcium supplements or have other known causes for hypercalcemia, such as hyperparathyroidism. A majority of our studied patients were of European white ancestry.

Sixty-six patients with non-LS and 5 with LS fulfilling these requirements were identified. Patients with LS were excluded from analysis due to the small number. As a control group, 150 normocalcemic non-LS patients were randomly selected from the same cohort. In all patients, calcium levels were routinely analyzed at first visit and revisits, with a frequency of every third month to every other year, with less frequent evaluations in stable patients. All patients in the control group disclosed normal calcium levels at every evaluation during a follow-up time of at least two years.

2.2. Parameters

Hypercalcemia was defined using laboratory reference values. For most patients this was defined as serum Ca^{2+} (s-Ca^{2+})>1.33 mmol/L (reference 1.15–1.33 mmol/L). Due to changes in reference values during the study period, it was defined as >1.34 for two and >1.35 for eleven patients. Patients were divided into three groups; patients with normal calcium values (controls), mild hypercalcemia (s-Ca^{2+} 1.34–1.4 mmol/L), severe hypercalcemia (>1.4 mmol/L).

Data on sex, age at sarcoidosis diagnosis, when SAHC was detected in relation to sarcoidosis diagnosis, chest X-ray at sarcoidosis diagnosis (Scadding's staging system was used for classification), *HLA-DRB1* alleles, treatment status (missing information in 2 out of 66 patients), s-ACE, plasma creatinine (p-creatinine), and EPM were retrieved. S–Ca^{2+} was recorded, p-creatinine and s-ACE were matched as close as possible to the date of collection. P-creatinine was also recorded at least 3 months before hypercalcemia occurred (missing data in 13 of 66 patients). Reference values used for creatinine were <90 μmol/L for female, <100 μmol/L for male. Five patients were on treatment with ACE inhibitors and were excluded from the analysis of ACE. EPM was defined as a positive biopsy from the affected organ or obvious symptoms/assessment from a specialist in the area. Hypercalciuria was not considered an EPM. Remaining signs of disease >2 years were defined as non-resolving disease, evaluated by chest X-ray, lung function test, presence of EPM, patient symptoms and laboratory signs of inflammation. For three of the controls, we were not able to retrieve data on resolving vs non-resolving disease. In addition, patients with hypercalcemia were also classified whether the sarcoidosis was progressive or stable when SAHC developed. Progressive disease was defined as deterioration of symptoms, impairment in chest radiological signs compared to previous assessment and/or systemic treatment required. Stable disease was defined as stable pulmonary manifestations without deterioration with no signs of inflammatory activity in laboratory parameters and/or no or minor chest radiological changes compared to previous assessment and/or no systemic treatment required.

2.3. HLA-typing

Genomic DNA was extracted from whole blood samples. For most patients, *HLA-DRB1* typing with 2-digit allelic resolution was determined using the polymerase chain reaction-sequence-specific primers (PCR-SSP) technique. For patients that were HLA-typed during the last five years the PCR-SSP technique was performed with an SSO DR low-resolution kit according to the manufacturers One Lambda's recommendations.

2.4. Statistical analysis

Differences between groups were analyzed with Fisher's exact test or Chi-square where appropriate. For correlation analysis, Spearman's rank correlation was used. The nominal p-value significance was set at <0.05. Moreover, to minimize the risk of type 1 error due to multiple testing of the *HLA-DBR1* alleles (n = 13), a Bonferroni correction p-value was set to determine statistical significance ($p < 0.05/13 = p < 0.0038$). P-values are presented uncorrected. Statistical analyses were performed with Graph Pad Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

Out of the 66 patients with SAHC, 35 (53%) carried the *HLA-DRB1*04* allele, compared to 49 of 150 normocalcemic controls (33%, $p < 0.01$) which was not statistically significant according to Bonferroni correction (p-value > 0.0038). A more pronounced proportion of patients carrying the allele *HLA-DRB1*04* was observed with increasing s-Ca^{2+}, 20 of 30 patients with s-Ca^{2+}>1.4 mmol/L carried the *HLA-DRB1*04* allele (67%, $p < 0.01$), see Table 1.

*HLA-DRB1*03* was less common in patients with SAHC (8%) compared to controls (23%, $p < 0.01$), see Fig. 1, but not statistically significant according to Bonferroni correction (p-value > 0.0038). No corresponding decrease of the proportion of patients carrying the *HLA-DRB1*03* allele was seen the more pronounced hypercalcemia.

No associations between other *HLA-DRB1* alleles and SAHC were detected.

Table 1
Comparisons of different hypercalcemic phenotypes.

	Normal calcium s-Ca^{2+}≤1.33	Mild hypercalcemia s-Ca^{2+}≤1.4	Severe hypercalcemia s-Ca^{2+}>1.4	p-value
*HLA-DRB1*04*	49 (33)	15 (42)	20 (67)	= 0.0021
Elevated p-creatinine	11 (7)	14 (39)	27 (90)	<0.001
Elevated ACE[a]	53 (35)	15 (43)	23 (88)	<0.001
EPM	67 (45)	23 (64)	20 (67)	<0.05

Data is presented as n (%).
EPM = extrapulmonary manifestations.
[a] Five patients were on ACE-inhibitors and therefore excluded.

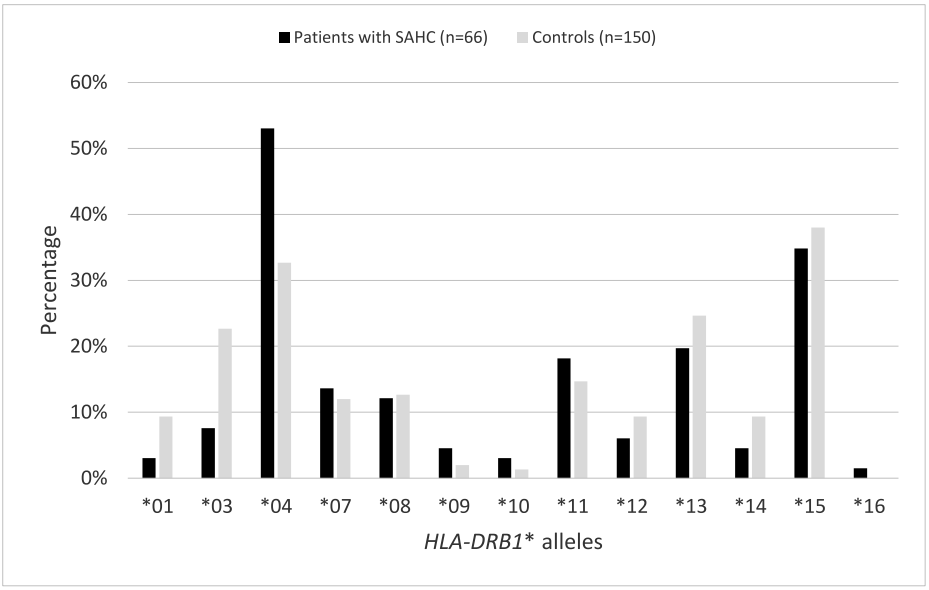

Fig. 1. Frequency of *HLA-DRB1* alleles amongst all SAHC-cases (n = 66) compared to the control group (n = 150).

P-creatinine, before development of hypercalcemia, was elevated in 21% of all patients. However, the elevation was mostly mild, 6 out of 11 patients had p-creatinine values below 110 μmol/L, and only 2 disclosed values above 150 μmol/L.

P-creatinine, recorded at the time for hypercalcemia, was elevated in 62% of all patients with SAHC; 39% of SAHC-patients with mild hypercalcemia (s-Ca^{2+} 1.34–1.4 mmol/L), increasing to 90% in the group with s-Ca^{2+}>1.4, but in only 7% of controls, $p < 0.001$. There was a positive correlation between s-Ca^{2+} and p-creatinine (correlation coefficient 0.65 in all SAHC-patients) see Fig. 2. In patients with mild hypercalcemia, 43% disclosed an elevated s-ACE, and 88% in the group with s-Ca^{2+}>1.4 whereas 35% of controls had elevated s-ACE, p <

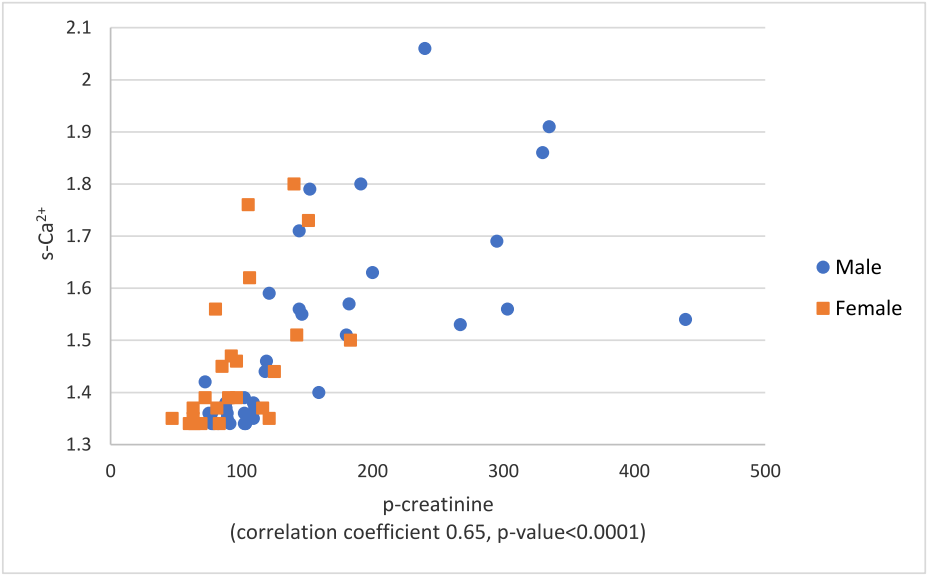

Fig. 2. S–Ca^{2+} (mmol/L) in relation to p-creatinine (reference values < 90 μmol for women, <100 μmol for men) for all patients with SAHC (n = 66).

0.001. EPM were present in 64% of patients with mild hypercalcemia, and 67% in the group with s-Ca^{2+}>1.4 whereas 45% of controls disclosed EPM, $p < 0.05$, see Table 1.

The mean duration between sarcoidosis diagnosis and detection of hypercalcemia was 1.39 years, with a spread from around diagnosis to 7 years. Most patients, 57 out of 64, were without treatment with corticosteroids/other immunosuppressants when hypercalcemia occurred, and 60 out of 66 patients were considered having a progressive disease at time for hypercalcemia. The majority (92%) of SAHC patients had a non-resolving disease after two years, compared to 78% of controls ($p < 0.05$). No difference was observed in age at sarcoidosis diagnosis, sex or Scadding stage between patients with SAHC and controls (see Table 2).

Combining the examined parameters, we found that only 1 of 30 patients with s-Ca^{2+}>1.4 had neither EPM, ACE elevation nor *HLA-DRB1*04*.

4. Discussion

SAHC is a challenging clinical problem as it can result in severe morbidity and often needs long-term treatment. Identifying patients who may be at higher risk of developing SAHC can help intervene early and closely monitor them.

In this study, we found that the proportion of patients with *HLA-DRB1*04* increased the more pronounced hypercalcemia. The frequency of *HLA-DRB1*04* in our control group was comparable to the frequency previously reported in Swedish sarcoidosis patients with non-LS [3].

Furthermore, the group of patients with SAHC more often had elevated p-creatinine and s-ACE levels, disclosed a higher frequency of EPM and more often had a non-resolving disease than normocalcemic patients, especially in patients with more severe hypercalcemia. We also found that SAHC can develop years after diagnosis. Our results are in line with previous findings of a connection between *HLA-DRB1*04* and EPM, and *HLA-DRB1*03* being protective for both EPM and non-resolving disease [3,6]. We did not see any connection between neither *HLA-DRB1*14* nor *HLA-DRB1*15* and SAHC, even though these HLA-haplotypes increase the risk for a prolonged disease course [3]. As we used a 2-digit allelic resolution method for HLA-typing, we are unable to tell whether there is an association between *HLA-DRB1** on the 4-digit level and SAHC. Rossman et al. observed an association with *HLA-DRB1*1101* and SAHC in white Americans [5]. Later, the same authors reported a connection between *HLA-DRB1*1101*, SAHC and exposure to insecticide [26]. We lack data for environmental exposures in our cohort. However, we believe that exposure to insecticides in our cohort is low, as the majority of patients were recruited from urban areas. Additionally, we cannot discard the possibility of other environmental factors interacting with *HLA-DRB1*04* and implicated in the onset of sarcoidosis with hypercalcemia.

Renal insufficiency can be a deleterious consequence of hypercalcemia, and we found that p-creatinine was related to s-Ca^{2+}, and was increased significantly in patients with s-Ca^{2+}>1.4. Our study's percentage of patients with renal insufficiency and SAHC, 62%, is higher than 40% reported in a previous study from the US [12]. This can have several explanations, e.g., differences in reference values. Also, whether ethnicity and sex have an impact on kidney function in sarcoidosis has, to our knowledge, not been investigated, but it may be a possibility that also these factors have influenced the results as our registry mostly consists of white males. Renal dysfunction in sarcoidosis can be caused by interstitial nephritis and as we did not take kidney biopsies, we cannot rule out that hypercalcemia in some of our patients actually resulted from renal sarcoidosis or another disease causing renal function impairment [2,11].

*HLA-DRB1*04* is connected to EPM, suggesting a higher burden of granulomas. Thus, a possible mechanism for SAHC association with *HLA-DRB1*04* is 1-alpha hydroxylase activity and/or production of PTHrp in the granulomas. Supporting this hypothesis is our finding that patients with SAHC more often had an elevated s-ACE compared to patients without SAHC, as ACE levels are believed to indicate the granuloma burden [27]. Furthermore, we show that most patients with SAHC have a non-resolving disease, and interestingly serum 1,25-(OH)2 vitamin D3 levels has been positively associated with a non-resolving disease [21].

However, 1,25-(OH)2 vitamin D3 can also be normal in patients with SAHC. Subsequently, other possible mechanisms may explain SAHC, at least in some patients. For instance, several single nucleotide polymorphisms (SNPs) of importance for calcium homeostasis have been identified [28,29]. To our knowledge, no one investigated a possible association between *HLA-DRB1*04* and calcium regulating genes, but a Japanese study reported an association between specific HLA alleles and peak bone mass [30].

SAHC has previously been reported to occur more frequently in males and white patients over 40 years old [10,31]. We found no difference in age or sex. However, it must be stressed, that our study group consisted mainly of male patients of European white ancestry and over 40 years old [10], i.e. similar characteristics as most sarcoidosis patients in Sweden [32]; thus, differences in age and sex may have been undetected.

Limitations of the study. Although our register contains a relatively large number of patients, further subgrouping will result in relatively few study patients, and therefore we might have missed minor differences between patients with SAHC and those without. We used a low-resolution method for HLA-typing, that is, a 2-digit resolution, and thus we could have missed differences on the 4-digit level. Moreover, we did not actively screen for EPM and therefore these might be underrated. Kidney biopsies are not routinely performed in our studied group of patients, and we may therefore have missed other causes for hypercalcemia. The study design was retrospective, and a local registry and the medical record were used to identify patients with hypercalcemia. S–Ca^{2+} was analyzed with varying frequencies which can have influenced the time to first detection. It is also possible that patients with hypercalcemia were missed if this information was not clearly stated in the registry or the medical record. However, we found 71 patients with SAHC of 1229 patients with sarcoidosis, equal to nearly 6%, which is a

Table 2
Clinical characteristics of patients with hypercalcemia and controls.

	Normal calcium s-Ca^{2+}≤1.33	All patients with hypercalcemia	Mild hypercalcemia s-Ca^{2+}≤1.4	Severe hypercalcemia s-Ca^{2+}>1.4
Subjects (n)	150	66	36	30
Age[a], years	46 (26–78)	45 (23–78)	43 (23–67)	47 (26–77)
Sex, male n (%)	96 (64)	42 (64)	23 (64)	19 (63)
Scadding stage 0-IV	4/44/76/19/7	6/15/38/4/3	3/10/18/2/3	3/5/20/2/0
Non-resolving n (%)[b]	115 (78)	61 (92)	35 (97)	26 (87)
Duration to SAHC years[c]	0	1 (0–7)	1 (0–9)	1 (0–7)

SAHC=Sarcoidosis associated hypercalcemia.

[a] Age, years at time for sarcoidosis diagnosis, values are medians (minimum to maximum).

[b] For 3 subjects in the control group information was lacking on resolving/non-resolving disease.

[c] Data from 62 out of 66 patients with hypercalcemia (missing data in 2 patients with mild hypercalcemia and 2 patients with severe hypercalcemia). Values are medians (minimum to maximum).

frequency comparable with previous reports [6,12].

The major strength of our study is that it is based on a sizeable well-characterized patient group, composed of European white ancestry individuals, specifically Nordic descent, all with non-LS sarcoidosis phenotype coupled with genotype data.

In conclusion, sarcoidosis patients should be repeatedly screened for hypercalcemia. It can develop several years after diagnosis, and it seems reasonable to be extra attentive with patients who carry the *HLA-DRB1*04* allele and patients with EPM, elevated ACE, and/or non-resolving disease. As our study population consisted mainly of Nordic patients, the results cannot be readily transferable to individuals of other ancestries.

In the future, as we continue to include more patients, we plan to study more in detail genes of importance for calcium metabolism. Results on the role of calcium and vitamin D supplements in the development of SAHC are conflicting [12,33,34], and therefore, we also plan to study if certain HLA-types predispose for the development of hypercalcemia during such treatment.

Through the MESARGEN consortium, (https://mesargen.wordpress.com), genome-wide data based on the Illumina GSA-MD SNP-array and phenotypic information is collected from population-based cohorts of sarcoidosis patients in several countries, we will be able to study *HLA-DRB1* alleles in relation to SAHC in a large-scale and in other ethnic groups.

Ethics approval and consent to participate

All included patients had signed a written consent form and approval was granted from the regional ethical review board in Stockholm.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Joanna Werner: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft, Writing – Review and Editing. **Anders Eklund and Tomoko Iseda:** Investigation, Writing – Review and Editing. **Johan Grunewald, Natalia Rivera and Pernilla Darlington;** Conceptualization, Methodology, Formal analysis, Writing – Review and Editing. **Susanna Kullberg**: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Review and Editing. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

SAHC	Sarcoidosis associated hypercalcemia
HLA	Human leukocyte antigen
LS	Löfgren's syndrome
Non-LS	Non-Löfgren's syndrome
EPM	Extrapulmonary manifestations
ACE	Angiotensin-converting enzyme
PTHrp:	Parathyroid hormone-related peptide
WASOG	World Association of Sarcoidosis and other Granulomatous disorders

References

[1] G.W. Hunninghake, et al., ATS/ERS/WASOG statement on sarcoidosis. American thoracic society/European respiratory society/world association of sarcoidosis and other granulomatous disorders, Sarcoidosis Vasc. Diffuse Lung Dis. 16 (2) (1999) 149–173.
[2] E.D. Crouser, et al., Diagnosis and detection of sarcoidosis. An official American thoracic society clinical practice guideline, Am. J. Respir. Crit. Care Med. 201 (8) (2020) e26–e51.
[3] J. Grunewald, et al., Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients, Respir. Res. 11 (2010) 25.
[4] P. Darlington, et al., HLA-DRB1* alleles and symptoms associated with Heerfordt's syndrome in sarcoidosis, Eur. Respir. J. 38 (5) (2011) 1151–1157.
[5] M.D. Rossman, et al., HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites, Am. J. Hum. Genet. 73 (4) (2003) 720–735.
[6] P. Darlington, et al., HLA-alleles associated with increased risk for extra-pulmonary involvement in sarcoidosis, Tissue Antigens 83 (4) (2014) 267–272.
[7] M. Berlin, et al., HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis, Am. J. Respir. Crit. Care Med. 156 (5) (1997) 1601–1605.
[8] J. Grunewald, A. Eklund, O. Olerup, Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients, Am. J. Respir. Crit. Care Med. 169 (6) (2004) 696–702.
[9] G.T. Harrell, S. Fisher, Blood chemical changes IN BOECK'S sarcoid with particular reference to protein, calcium and phosphatase values, J. Clin. Invest. 18 (6) (1939) 687–693.
[10] R.P. Baughman, et al., Clinical characteristics of patients in a case control study of sarcoidosis, Am. J. Respir. Crit. Care Med. 164 (10 Pt 1) (2001) 1885–1889.
[11] M. Mahevas, et al., Renal sarcoidosis: clinical, laboratory, and histologic presentation and outcome in 47 patients, Medicine (Baltim.) 88 (2) (2009) 98–106.
[12] R.P. Baughman, et al., Calcium and vitamin D metabolism in sarcoidosis, Sarcoidosis Vasc. Diffuse Lung Dis. 30 (2) (2013) 113–120.
[13] J.S. Adams, et al., Isolation and structural identification of 1,25-dihydroxyvitamin D3 produced by cultured alveolar macrophages in sarcoidosis, J. Clin. Endocrinol. Metab. 60 (5) (1985) 960–966.
[14] J.S. Adams, et al., Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis, J. Clin. Invest. 72 (5) (1983) 1856–1860.
[15] A.S. Dusso, et al., Extrarenal production of calcitriol in normal and uremic humans, J. Clin. Endocrinol. Metab. 72 (1) (1991) 157–164.
[16] A.S. Dusso, et al., gamma-Interferon-induced resistance to 1,25-(OH)2 D3 in human monocytes and macrophages: a mechanism for the hypercalcemia of various granulomatoses, J. Clin. Endocrinol. Metab. 82 (7) (1997) 2222–2232.
[17] P.J. Tebben, R.J. Singh, R. Kumar, Vitamin D-mediated hypercalcemia: mechanisms, diagnosis, and treatment, Endocr. Rev. 37 (5) (2016) 521–547.
[18] O.P. Sharma, Hypercalcemia in granulomatous disorders: a clinical review, Curr. Opin. Pulm. Med. 6 (5) (2000) 442–447.
[19] K. Hamada, et al., Ionized calcium and 1,25-dihydroxyvitamin D concentration in serum of patients with sarcoidosis, Eur. Respir. J. 11 (5) (1998) 1015–1020.
[20] R.S. Mason, et al., Vitamin D conversion by sarcoid lymph node homogenate, Ann. Intern. Med. 100 (1) (1984) 59–61.
[21] D. Kavathia, et al., Elevated 1, 25-dihydroxyvitamin D levels are associated with protracted treatment in sarcoidosis, Respir. Med. 104 (4) (2010) 564–570.
[22] S. Falk, et al., Hypercalcemia as a result of sarcoidosis with normal serum concentrations of vitamin D, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 13 (11) (2007) Cs133–136.
[23] C. Alberts, H. van den Berg, Calcium metabolism in sarcoidosis. A follow-up study with respect to parathyroid hormone and vitamin D metabolites, Eur. J. Respir. Dis. 68 (3) (1986) 186–194.
[24] D.H. van Raalte, et al., Sarcoidosis-related hypercalcaemia due to production of parathyroid hormone-related peptide, BMJ Case Rep. 2015 (2015).
[25] H.J. Zeimer, et al., Parathyroid-hormone-related protein in sarcoidosis, Am. J. Pathol. 152 (1) (1998) 17–21.

[26] M.D. Rossman, et al., Hla and environmental interactions IN sarcoidosis, Sarcoidosis Vasc. Diffuse Lung Dis. 25 (2) (2008) 125–132.
[27] S. Gilbert, et al., Amounts of angiotensin-converting enzyme mRNA reflect the burden of granulomas in granulomatous lung disease 148 (2) (1993) 483–486.
[28] C.M. O'Seaghdha, et al., Common variants in the calcium-sensing receptor gene are associated with total serum calcium levels, Hum. Mol. Genet. 19 (21) (2010) 4296–4303.
[29] L. Xu, S.L. Lin, C.M. Schooling, A Mendelian randomization study of the effect of calcium on coronary artery disease, myocardial infarction and their risk factors, Sci. Rep. 7 (2017) 42691.
[30] S. Tsuji, et al., HLA-A*24-B*07-DRB1*01 haplotype implicated with genetic disposition of peak bone mass in healthy young Japanese women, Hum. Immunol. 59 (4) (1998) 243–249.
[31] L.E. Siltzbach, et al., Course and prognosis of sarcoidosis around the world, Am. J. Med. 57 (6) (1974) 847–852.
[32] E.V. Arkema, et al., Sarcoidosis incidence and prevalence: a nationwide register-based assessment in Sweden, Eur. Respir. J. 48 (6) (2016) 1690–1699.
[33] M.J. Bolland, et al., Randomised controlled trial of vitamin D supplementation in sarcoidosis, BMJ Open 3 (10) (2013), e003562.
[34] L.S. Kamphuis, et al., Calcium and vitamin D in sarcoidosis: is supplementation safe? J. Bone Miner. Res. 29 (11) (2014) 2498–2503.