Ätiopathogenese der Sarkoidose

Martin Brutsche
Search for hints...

1. Is it a genetic disease?
2. Is it infection?
3. Is it induced by specific exposures to organic/anorganic antigens?
4. Is it Autoimmunity
5. Summary of confusion
High-Density Genetic Mapping Identifies New Susceptibility Variants in Sarcoidosis Phenotypes and Shows Genomic-driven Phenotypic Differences

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Abstract

Rationale: Sarcoidosis is a multisystem disease of unknown cause. Löfgren’s syndrome (LS) is a characteristic subgroup of sarcoidosis that is associated with a good prognosis in sarcoidosis. However, little is known about its genetic architecture or its broader phenotype, non-LS sarcoidosis.

Objectives: To address the genetic architecture of sarcoidosis phenotypes, LS and non-LS.

Methods: An association study in a white Swedish cohort of 384 LS, 664 non-LS, and 2,086 control subjects, totaling 3,134 subjects using a fine-mapping genotyping platform was conducted. Replication was performed in four independent cohorts, three of white European descent (Germany, n = 4,975; the Netherlands, n = 613; and Czech Republic, n = 521), and one of black African descent (United States, n = 1,657), totaling 7,766 subjects.

Measurements and Main Results: A total of 727 LS-associated variants expanding throughout the extended major histocompatibility complex (MHC) region and 68 non-LS-associated variants located in the MHC class II region were identified and confirmed. A shared overlap between LS and non-LS defined by 17 variants located in the MHC class II region was found. Outside the MHC region, two LS-associated loci, in ADCY3 and between CSMD1 and MCPH1, were observed and replicated.

Conclusions: Comprehensive and integrative analyses of genetics, transcription, and pathway modeling on LS and non-LS indicates that these sarcoidosis phenotypes have different genetic susceptibility, genomic distributions, and cellular activities, suggesting distinct molecular mechanisms in pathways related to immune response with a common region.

Keywords: genetic epidemiology of sarcoidosis; Löfgren’s syndrome; non-Löfgren’s syndrome; genome-wide associations; Immunochip
Figure 5. Distribution of cis-expression quantitative trait loci (eQTL) and regulatory single-nucleotide polymorphisms (SNPs) among associated confirmed variants of Löfgren’s syndrome (LS), non-LS, and both. Percentages are derived from the total number in each group, respectively. Genes enclosed in parentheses denote intergenic regions. HC = healthy control subjects; MHC = major histocompatibility complex; TF = transcription factor.
Linkage Analyses

• Susceptibility for sarcoidosis
  – Annexin A11 (GWAS >440‘000 SNPs in 499 German patients and 490 controls; Nat Genetics 2008)

• Löfgren vs. Non-Löfgren
  – IL7R: sarcoidosis & Löfgren (Genes Immun. 2009)
  – C-C chemokine receptor 5 gene: Female-specific association (J Mol Med 2008)
Butyrophilin-like 2 (BTNL2) is involved in co-stimulation of T-Lymphocytes due to similarities to B7-1.

Risk allel has truncated form resulting in increased activation of T-lymphocytes.

RR for sarcoidosis +60%
Antworten aus der Genetik

- Sarkoidose ist keine Erbkrankheit
- Genpolymorphismen für die Empfindlichkeit für die Sarkoidose
- Genpolymorphismen für die phänotypische Präsentation der Sarkoidose – Löfgren vs. Non-Löfgren
- Polymorphismen weisen zu Mechanismen der Zell-vermittelten Immunantwort und Antigen- & Proteinverarbeitung → Pathogenese
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Wie verläuft die Entzündung?

- Aktivierung der angeborenen, zell-vermittelten Immunantwort
  - TH1-Immunantwort
  - Abwehr von intrazellulären Keimen wie Mykobakterien, Propionibakterien

Iannuzzi et al. NEJM 2007
Tissue of sarcoidosis patients: ICH, ELISA
- SAA is highly expressed in granulomata in sarcoidosis and not so in other granulomatous disorders

BAL of sarcoidosis patients for functional studies, including in vitro-stimulation model
- SAA induces activation of macrophages via TLR-2
SAA is highly expressed in granulomas in sarcoidosis

Chen ES et al. AJRCCM 2010
SAA is highly expressed in granulomas in sarcoidosis. ...not so in other granulomatous disorders...
Comparison between TB and sarcoidosis

Whole-blood gene expression profiling, microRNA expression, and multiplex serum analytes

Very similar...!

Sarcoidosis is like TB, but without the presence of living microorganisms!?!
Fig. 1. Common and differential expression of genes, miRNAs, and serum analytes. Venn diagrams show the number of significantly (q < 0.01) differentially expressed genes, miRNAs, and cytokines (P < 0.01) between the disease groups and healthy controls. Red and green areas are disease-specific for TB and SARC, respectively; overlap in yellow represents differential expression in both diseases. Right shows the number of differentially expressed analytes in direct comparison between TB and SARC.
Mycobacterial catalase–peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis


Zhimin Song,¹ Lisa Marzilli,² Brian M. Greenlee,¹ Edward S. Chen,¹ Richard F. Silver,⁵ Frederic B. Askin,³ Alvin S. Teirstein,⁶ Ying Zhang,⁴ Robert J. Cotter,² and David R. Moller¹

• Limited proteomics in serum for poorly soluble/protease-resistant tissue antigens (i.e. properties of granuloma-inducing sarcoidosis tissue extracts)
  – Incl. Mycobacterium tuberculosis catalase–peroxidase (mKatG)

• Such tissue antigens found in 9/12 (75%) sarcoidosis tissues (3/22 (14%) in control tissues (p=0.0006)
Mycobacterium tuberculosis catalase–peroxidase (mKatG)


- mKatG-ICH positive in 5/9 (55%) sarcoidosis tissues (0/14 control tissues; p=0.004)
- IgG antibodies to recombinant mKatG detected in sera of 12/25 (48%) sarcoidosis patients (0/11 PPD- controls; p=0.006 & 4/10 PPD+ controls; p=0.72)
- Remnant mycobacterial catalase–peroxidase might be one target of the adaptive immune response driving granulomatous inflammation in sarcoidosis
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Significant heterogeneity $p<0.001$
Population density $p=0.04$ (negative correlation)

Medical services $p=0.43$
Air pollution $p=0.43$
Associations with different methods of agriculture

$p=0.004$

Deubelbeiss et al. Eur Respir J. 2010
Associations with industries (Co-inertia)

Deubelbeiss et al. Eur Respir J. 2010
Häufigkeit von CBD

• Sensibilisierung 2-20% der Be Exponierten
• Ca. 40% von Sensibilisierten entwickeln CBD
• Ca. 6-7% von Sarkoidosis Patienten waren in D und Israel in Wahrheit CBD
• 16-30% von Sarkoidosis Patienten sind/waren Be exponiert, von diesen sind zwischen 20-40% CBD
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Autoimmunity?

• PRO
  – Associations with HLA-types (Wahlström J et al. J Clin Invest 2007)
  – Presence of hypergammaglobulinemia (Hunninghake GW, Crystal RG. J Clin Invest 1981)

• CON
  – Compartimalization of immune response
  – Peripheral anergia of immune response
Proteomic Profiling Reveals Autoimmune Targets in Sarcoidosis

Antigen microarrays (3,072 protein fragments) to screen for IgG reactivity in 73 BAL samples from sarcoidosis, asthma & healthy subjects

131 targets verified on suspension bead arrays using 272 additional BAL and 141 paired sera

Reactivity to 4 antigens was analyzed in 22 unprocessed BAL samples from patients with fibrosis and 269 plasma samples from patients diagnosed with myositis

Anna Häggmark, Carl Hamsten, Emil Wiklundh, Cecilia Lindskog, Cecilia Mattsson, Eni Andersson, Ingrid E. Lundberg, Hans Grönlund, Jochen M. Schwenk, Anders Eklund, Johan Grunewald, and Peter Nilsson

Häggmark A et al. Am J Respir Crit Care Med 2015
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Table 5. Reactivity Comparisons

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency of Reactivity (%) (Number of Reactive Samples)</th>
<th>P Value (Adjusted P Value)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sarcoidosis</td>
<td>LS</td>
</tr>
<tr>
<td>ZNF688</td>
<td>71 (198)</td>
<td>71 (98)</td>
</tr>
<tr>
<td>MRPL43</td>
<td>12 (33)</td>
<td>14 (20)</td>
</tr>
<tr>
<td>NCOA2</td>
<td>6 (18)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>ARFGAP1</td>
<td>40 (112)</td>
<td>38 (53)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: LS = Lofgren syndrome; ZNF688 = zinc finger protein 688.
The number of reactive samples and their frequencies are shown per sample group with P values from comparative analysis of frequencies (Fisher) and intensity levels (Wilcoxon) shown with and without adjustment for multiple testing.

- Identification of 4 promising autoantigens
- Most patients had reactivity to either of these autoantigens
T-cell receptor–HLA-DRB1 associations suggest specific antigens in pulmonary sarcoidosis

J. Grunewald et al. ERJ 2016

Johan Grunewald¹, Ylva Kaiser¹, Mahyar Ostadkarampour¹, Natalia V. Rivera¹, Francesco Vezzi², Britta Lötstedt³, Remi-André Olsen², Lina Sylwan³, Sverker Lundin⁴, Max Käller⁴, Tatiana Sandalova⁵, Kerstin M. Ahlgren¹, Jan Wahlström¹, Adnane Achour⁵, Marcus Ronninger¹ and Anders Eklund¹

- Pulmonary sarcoidosis patients (n=43, incl. 26 HLA-DRB1*03⁺) → bronchoscopy & BAL
- TCR α and β chains of CD4+ T-cells were analysed by flow cytometry, DNA-sequenced, and 3D-molecular models of TCR-HLA-DRB1*03 complexes generated
TCR-Construct
TCR-chains, CDR-loops, HLA-DR-chains

Antigen-Pocket
Vimentin-derived peptide Vim$_{429-443}$ fits in pocket
Autoimmunity?

• Possible through molecular mimicry
  – Antigen hits the body
  – Incomplete elimination of proteins/peptides
  – Remnant misfolded and bacterial proteins might have resemblance to autoantigens
  – Persistance of immune response after clearance of infection

• Concept of autoimmunity needs to be revisited
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Summary

- Sarcoidosis is characterized by an activation of innate immunity and granuloma formation.

- It is likely that:
  - the immune response is induced by antigen(-s) – sometimes in a context of (airborne) infection
  - Remnant/aggregated antigens form the nidus for granuloma formation
  - Molecular mimicry might induce autoimmunity

- It is possible that:
  - Berylliosis is not another disease, but a sarcoidosis with known antigen