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Immunogenetics of Sarcoidosis

Amit Kishore and Martin Petrek*

***Abstract*—Sarcoidosis is a multi-facets disease. Along with environment, the role of genetic component in this immune-mediated granulomatous disorder is undisputable, irrespective of the tissue/ organ involved (lung, eyes, heart, etc.). Due to undefined etiology of this complex disorder, it has remained as challenge to control the disease progression. Further, identification of prognostic genetic maker for this disease at functional as well as non-functional level has remained intriguing. To understand the sarcoidosis susceptibility at genetic level, several molecular approaches, such as meta-analysis of GWAS, significant association of coding and rare variants, fine mapping of susceptible loci, gene networking and their associated mechanism, copy number variations, single nucleotide polymorphisms in miRNAs (mirSNPs) and epigenetic changes in case control studies across cohorts of different ethnic groups would reveal a better insight.**

***Keywords* — Sarcoidosis, immunopathogenesis, GWAS, non-HLA candidate genes, HLA complex, disease susceptibility**

I. Introduction

SARCOIDOSIS, a multisystem disorder of unknown etiology, is characterized by local noncaseating epithelioid granulomas in the involved organs [1,2]. The granulomas comprise of epithelioid cells, macrophages, and multinucleated giant cells surrounded by lymphocytes, mast cells, monocytes, and fibroblasts [3]. Immunohistochemical staining of sarcoid granulomas display majority of lymphocytes as $CD4^+$ T cells, however, periphery of the granuloma is composed of $CD4^+$ as well as $CD8^+$ T cells [4-6]. Sarcoidosis most frequently presents as pulmonary sarcoidosis (approximately 90 percent of patients). Other commonly involved tissues include skin, eyes, and lymph nodes. Pulmonary sarcoidosis is characterized by increased number of lymphocytes and macrophages in the alveoli.

Current opinion on the pathogenesis of sarcoidosis involves sequence of immunological reactions leading to granuloma formation. These events involve internalization of the foreign antigen(s), its further processing and presentation by antigen presenting cells (APCs) such as dendritic cells or macrophages, acquiring T cell immunity through the trimolecular complex (MHC/peptide/ TCR) interaction, generation of specific T-effector cells, activation of macrophages, and induction of granuloma formation [7,8]. The initial lesions within the pulmonary system are predominant in $CD4^+$ T cell alveolitis that upon T-cell receptor (TCR) activation initiate an inflammatory response. This potentiates activated macrophages and type 1 T helper cells to release varieties of cytokines, including tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), interleukin 2 (IL-2), and growth factors resulting to development of noncaseating granuloma [9-13].

Sarcoidosis has a clear genetic component and the evidences, such as occasional occurrence of familial sarcoidosis or clinical heterogeneity of sarcoidosis, was reviewed earlier [7, 14-25]. As the above described immune and inflammatory reactions are central to sarcoidosis pathogenesis, the genes encoding antigen presentation and recognition molecules including HLA, cytokines and receptors, etc., are among those which are mostly implicated as the genetic factors of disease (susceptibility/ protection/ clinical course). These were mostly deciphered using traditional case-control genetic association studies. The progress which can be further stimulated by applications of novel methodologies is also dependent on the availability of a disease model. Recent efforts, based on advanced methods such as genome scans and genome wide association studies (GWAS) not only confirm some of the previously implicated genes, but also bring along novel, sometimes "non-immune" candidate loci. This review will, therefore, focus on characterisation of the role of immune genes polymorphism in development of sarcoidosis and in modification of disease clinical course.

II. Immunopathogenesis of Sarcoidosis

The antigens responsible for initiation of sarcoid immune response have remained elusive [26]. The epidemiologic association with infectious agents, most frequently with mycobacteria (*Mycobacterium tuberculosis*) and propionibacteria (*Propionibacterium acnes, P. granulosum*) has long been suspected as possible causes of sarcoidosis [27-30]. However, it is clear that immupathomechanisms of sarcoidosis are multifactorial, i.e. are combination of host

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Amit Kishore is with Laboratory of Immunogenomics and Immunoproteomics, Institute of Molecular and Translational Medicine (IMTM), Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic (e-mail: amitkishore.bio@gmail.com).

Martin Petrek (*Corresponding author) is with Laboratory of Immunogenomics and Immunoproteomics, Institute of Molecular and Translational Medicine (IMTM), Faculty of Medicine and Dentistry, Palacky University, I. P. Pavlova str. 6, CZ-77520 Olomouc, Czech Republic. Tel: ++420 58 844 2285, Fax: ++420 58 541 5116 and Institute of Clinical and Molecular Pathology, University Hospital, Olomouc, Czech Republic (e-mail: martin.petrek@fnol.cz).

genetic background, the status of the host immune system, and the exposure itself.

A. GWAS for identification of loci associated with sarcoidosis

Genome Wide Association Studies (GWAS) have been carried out to investigate the genetic basis of complex disease such as Sarcoidosis. Since 2008, there have been six GWAS reported in sarcoidosis, five in European populations and one in African-Americans. These studies have provided novel insights for identification of important genomic regions including rare genetic variants influencing susceptibility to sarcoidosis with genome-wide significance. GWAS in sarcoidosis have indicated several candidate loci, majorly at autosomal chromosomes 6, 10, 11 and 12 to be associated with sarcoidosis (Figure 1, table 1). These include genes such as *Annexin A11* (*ANXA11*) [31] and *butyrophilin-like 2* (*BTNL2*) [32], which were later on confirmed by replication studies (Table 1). Novel loci, underlying variation and most promising candidates for the underlying risk gene(s) in the associated region includes 12q13.3–q14.1 (rs1050045 in the 3'-UTR of *OS9*), 11q13.1 (rs479777 in *CCDC88B*), 6p21.32 (rs715299 in *NOTCH4*) and 10p12.2 (rs1398024 in *C10orf67*) (Table 1). *NOTCH4* gene, which encodes a member of the Notch family, is involved in regulating the activity of T cell immune response and is located in proximity of MHC class II. In the intron region of *NOTCH4*, a novel variation (rs715299) having significant association with sarcoidosis was identified [33]. This association was determined to be independent of other neighboring MHC genes including *BTNL2* and it, therefore, represents an attractive biological candidate for future studies.

In an interesting study for identifying ancestry-linked genes predisposing to sarcoidosis at genome-wide scale, ancestry scan was conducted in African–Americans using a map of 1,384 marker panel with an average spacing of 2.9 cM [34]. They reported a highly significant ancestry association with sarcoidosis at marker rs11966463 on chromosome 6p22.3. Other marker includes rs1462906 on 8p12 and rs7919137 on chromosome 10p11.22.

B. Role of non-HLA candidate genes in sarcoidosis

Studies on genetic polymorphisms of immune genes other than HLA have suggested several variants as plausible markers for sarcoidosis as a whole or for certain subgroups of patients. However, prior to considering them as genetic biomarkers, it must be acknowledged that susceptibility factor for few of these variations were confined to a single population only and were not replicated in other populations. For example, the association between sarcoidosis and a SNP (C5507G; Pro1827Arg) in the *Complement receptor 1* gene (*CR1*, *1q32*) has been reported only once in Caucasians of Italian descent [35] but was not replicated in Czech and Dutch patients [36] (Table 2a). Similarly, G/C variation in intron-4 and $(GT)_n$ allele 2/3 variation in the 5'- region of *solute carrier protein A11A1* (*SLCA11A1*, formally known as *NRAMP1*) gene associated with sarcoidosis in Turkish population [37] was not replicated in African Americans [38], Polish [39] and Greek patients [40]. However, other associations have been consistently replicated and verified across populations. These includes genetic variants in chemokine receptor genes, namely *CCR2* (Val64Ile), B-7 family co-stimulatory molecule *BTNL-2* (rs2076530), molecular chaperon *HSPA1L* (+2437 CC genotype, C allele), tans-membrane receptor protein encoding *CFTR* (Arg75Gln), *ANXA11* (Arg230Cys, nominated by GWAS) [31], *CC10* (A allele), *VDR* (B allele), *NOD2* (His496Leu, Arg334Trp, Asp382Glu and Ala612Thr), *ACE* (Pro1199Leu), *TGF* (59941G, 4875A and 17369C alleles) and immune related responsive genes, such as *IFN-γ* (551 T/G), *IL-18* (genotypes -670CA and 656 GT), *TLR4* (Asp299Gly and Thr399Ile), *NFkBα* (-297T allele, -827T allele). In this context, it is important to perform investigations as per the standard practice of genetic association studies published in statements such as STREGA [41,42], or STREIS [43], which is specific for immunogenetics.

C. Human Leukocyte Antigens in sarcoidosis

The first descriptions towards relationship between sarcoidosis and immune genes come from 1980 ś, during the era of studying association between HLA antigens and immune / autoimmune disorders through serological typing using microlymphocytotoxicity test. The earliest studied HLA types were HLA-A, -B alleles along with their haplotypes [44, 45] and the first immunogenetic markers identified for sarcoidosis were HLA-A1, B8, and DR3 [14]. Further, advancement in tissue (HLA)-typing with molecular (DNA based) techniques, the observed associations among them became more stringent with significant increase in their number (Table 2b). Genetic susceptibility to sarcoidosis has been most closely linked with the major histocompatibility complex (MHC) comprising of HLA class I (HLA-A, -B, and -C) and class II (HLA-DP, -DQ, and -DR). Among them, HLA class II and its HLA-DRB1 and DQB1 alleles are predominantly shown to be associated with sarcoidosis (Table 2b). Studies have suggested that several alleles such as, HLA-DPB1*04:02 (Finnish population), HLA-DQB1*0201 (African Americans, British and Dutch white patients), DQB1*0501 (UK, Dutch and Japenese patients), DRB1*01 (Italian and Czech populations) and DRB1*04 (Finnish population) are protective towards sarcoidosis, whereas, DQB1*0602 (African Americans, Dutch caucasian), DRB1*1101 (African-Americans and Caucasians), HLA-DRB1*1501 (Finnish, Scandinavian, African-Americans and Caucasians) and HLA-DRB3*0101 (Japanese) confer susceptibility/ progression of the disease. However, HLA-DRB1*0301 (Scandinavian subjects) have been associated with acute sarcoidosis/ Löfgren's syndrome (LS). Besides, haplotypic combinations among HLA-DR and HLA-DQ alleles, such as HLA-DRB1*1501-DQB1*0602, HLA-DRB1*04-DQB1*0301, HLA-DRB1*0301-DRB3*0101 have been suggested as risk factor for pulmonary sarcoidosis, whereas, HLA-DRB1*0401-DPB1*0401 was found to be enriched in the patients with resolving disease (Table 2b).

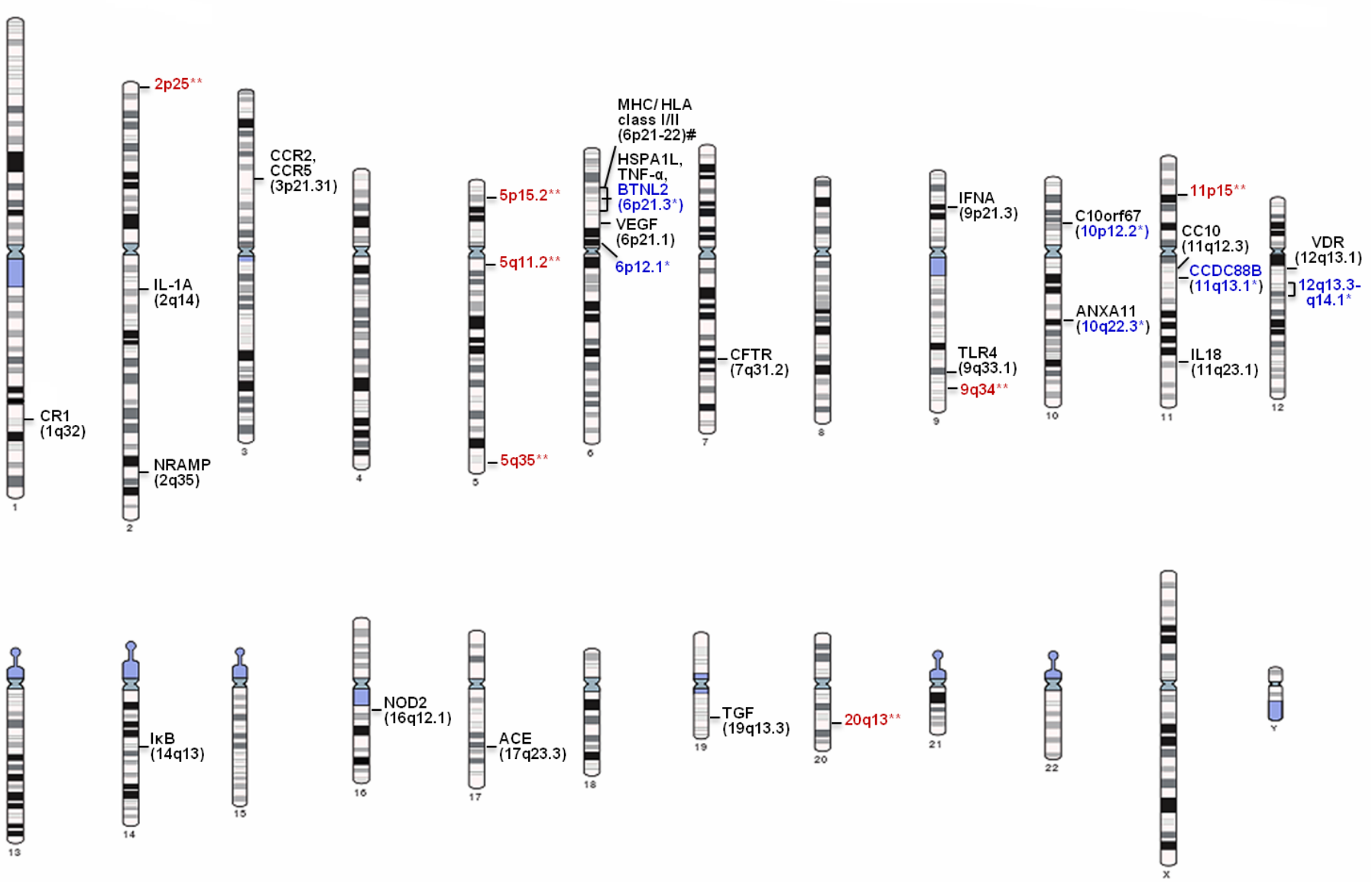

Fig. 1. Representation of loci from genome wide association studies (GWAS)* (blue colored), non-HLA candidate genes and fine mapping of susceptibility** (red colored) associated with Sarcoidosis. Major alleles of MHC/ HLA variants# are discussed in Table 2b.

TABLE 1

GWAS IDENTIFIED LOCI ASSOCIATED WITH SARCOIDOSIS IDENTIFIED USING GENOME-WIDE GENOTYPING PLATFORMS*

Disease/Trait; Platform [QC passed SNPs]	Initial Sample Size	Replication Sample Size	Region; reported Gene(s)	Strongest SNP-Risk Allele	p-value/ OR or beta-coeffi / [95% CI]
Genome-wide association analysis reveals 12q13.3-q14.1 as new risk locus for sarcoidosis [71]					
Sarcoidosis; Affymetrix 6.0 Human GeneChip [677,619]	637 cases, 1,233 controls in German population	3,121 cases, 4,284 controls- European ancestry	12q13.3–q14.1; *OS9*, 3'-UTR	rs1050045	9.22 x 10^{-8}/ 1.24 (risk factor)
A novel sarcoidosis risk locus for Europeans on chromosome 11q13.1 [72]					
Sarcoidosis; Affymetrix 6.0 Human GeneChip [1,294,967]	564 cases and 1,575 controls in German population	3,080 cases and 3,659 controls in German population	11q13.1; *CCDC88B, KCNK4, PRDX5, BAD, GPR137*	rs479777	2.68 x 10^{-18}/ 1.18 [1.18-1.43]
Genome-Wide Association Study of African and European Americans Implicates Multiple Shared and Ethnic Specific Loci in Sarcoidosis Susceptibility [33]					
Sarcoidosis; Illumina Omni1-Quad array [864,829]	818 cases and 1,088 controls- African Americans; 518 cases and 3,687 controls- European Americans	455 cases and 557 controls- African Americans; 442 cases and 2,284 controls- European Americans	*NOTCH4.* Also replicated *HLA-DRA, HLA-DRB5, HLA-DRB1, BTNL2,* and *ANXA11*	rs715299,	$P_{AA\text{-}meta}$ = 6.51 x 10^{-10}
A genome-wide association study reveals evidence of association with sarcoidosis at 6p12.1 [73]					
Sarcoidosis; Affymetrix 100k Human GeneChip [97,088]	381 cases and 392 controls in German population	1,582 cases and 1,783 controls in German population	6p12.1; *BAG2, C6orf65, KIAA1586, ZNF451* and *RAB23*	rs10484410	2.64 x 10^{-4}/ 1.72 [1.29-2.27]
Genome-wide association study identifies *ANXA11* as a new susceptibility locus for sarcoidosis [31]					
Sarcoidosis; Affymetrix Genome-Wide Human SNP Array 5.0 [375,771]	499 cases and 490 controls in German population	1,649 cases, 1,832 controls in German population	10q22.3; *ANXA11*	rs2789679	3 x 10^{-13}/ 0.6 [0.52–0.69]
				rs7091565	1 x 10^{-15}/ 1.26 (1.09–1.47)
				rs1049550	1 x 10^{-12} / 0.62 [0.54–0.71]
Genome-wide association analysis in Sarcoidosis and Crohn's disease unravels a common susceptibility locus on 10p12.2 [74]					
Crohn's disease (CD) and sarcoidosis (SA) (both); Affymetrix 100k Human GeneChip [83,360]	382 CD cases, 398 SA cases, 394 controls in German population	660 CD cases, 657 SA cases, 1,091 controls in German population	10p12.2; *C10orf67*	rs1398024	4.24 x 10^{-6} / 0.81 [0.69–0.96]

Abbreviations: *Coiled-coil domain containing 88B (CCDC88B), Osteosarcoma amplified 9 (OS9), Notch homolog protein 4 (NOTCH4).* *Figure 1- for chromosomal localization.

TABLE 2A
NON-HLA CANDIDATE GENE VARIANTS AND SARCOIDOSIS

Gene	Chromosomal location	Variation associated	Clinical Significance	Ref.
CR1	1q32	5507 GG genotype; Pro1827Arg	Susceptibility factor†	[35]
IL-1A	2q14	- 889 CC genotype; C allele	Susceptibility factor ††	[75]
NRAMP1/ SLCA11A1	2q35	$(CA)_n$ repeat in 5'- proximal region	Protective effect (P = 0.014)	[38]
		INT4 G/C	Association with Sarcoidosis†††	[37]
		5'-$(GT)_n$ allele 2/3	Association with Sarcoidosis†††	[37, 39]
CCR2	3p21.31	Val64Ile polymorphism	64Ile lower risk to Sarcoidosis and Löfgren's syndrome (LS) association	[76-79]
CCR5	3p21.31	CCR5Delta 32 allele	Association Refuted with haplotype analysis and larger sample.	[79, 80]
VEGF	6p21.1	+813 CT; TT genotypes and T allele	Protective effect	[81, 82]
BTNL2	6p21.3	rs2076530 (1078 A/G; Ser360Gly)	A allele and the AA genotype as risk factor	[32, 55, 57, 83-85]
HSPA1L	6p21.33	+2437 CC genotype and C allele	Susceptibility to Sarcoidosis and LS	[86]
TNF-α	6p21.33	rs1800629 (-308G/A)	Association of -308*A allele in patient with LS	[46-52]
		-1031 (P= 0 006) and -863 (P= 0.042)	Markers for onset of Sarcoidosis	[87]
C4	6p21.33	Copy number variations for isotypic forms of *C4* gene (*C4A* and *C4B*)	*C4A* deficiencies were associated with sarcoidosis, but *C4A* and *C4B* variations did not differ between resolved and persistent patient groups	[67]
CFTR	7q31.2	Arg75Gln	Predispose to the development of sarcoidosis	[88, 89]
IFN-γ	9p21.3	IFNA17 polymorphism (551 T/G) and IFNA10 [60A] -IFN17 [551G] haplotype	Associated with susceptibility to sarcoidosis	[90]
TLR4	9q33.1	Asp299Gly and Thr399Ile	Associated with chronic course of disease	[91]
ANXA11	10q22.3	rs1049550 (Arg230Cys)	Susceptibility in German, European, African Americans and European Americans	[31, 92-94]
		rs61860052, rs4377299	Susceptibility in African Americans	[94]
CC10/ SCGB1A1	11q12.3	A allele	Associated with disease progression up to 3 yr follow-up	[95]
IL-18	11q23.1	Genotypes -607CA and 656 GT	Significantly associated with serum levels of IL-18 in patients with sarcoidosis	[96, 97]
VDR	12q13.1	B allele	*Bsm*I allele elevated in sarcoidosis patients; genetic risk factor	[98, 99]
IκBα	14q13	-297T allele; allele -827T in stage II	Associated with disease progression	[100]
NOD2 / CARD15	16q12.1	His496Leu, Arg334Trp, Asp382Glu, Ala612Thr	Pathogenic to early-onset of Sarcoidosis	[101]
ACE	17q23.3	Pro1199Leu (rs121912703)	Elevated level of serum ACE can be helpful in diagnosis and to monitor Sarcoidosis	[102]
		I/D gene polymorphism	Increased risk for ID and DD genotypes. DD genotype is associated with poor prognosis and not significant for sarcoidosis susceptibility	[103-108]
TGF	19q13.3	TGF-beta2 59941 G, TGF-beta3 4875 A and 17369 C alleles	Predilection for pulmonary fibrosis development	[109]

No association was reported by [36]†; [110]††; [38-40]†††. ***Abbreviations:*** *Angiotensin-converting enzyme (ACE), Annexin A11 (ANXA11), Butyrophilin-like 2 (MHC class II associated) (BTNL2), C-C chemokine receptor 2 (CCR2), C-C chemokine receptor 5 (CCR5), CC-chemokine receptor 2 (CCR2), Clara cell 10 kD prt (CC10), Complement receptor 1 (CR1), Complement protein C4 (C4), Cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) (CFTR), Heat shock 70kDa protein 1-like (HSPA1L), IL-4 receptor (IL4R), Inhibitor κB-α (IκBα), Interferon-α (IFN-A), Interleukin-1 α (IL-1A), Interleukin-18 (IL-18), Major histocompatibility complex (MHC), Natural resistance associated macrophage protein (NRAMP1) also known as solute carrier protein 11A1 (SLC11A1), Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), Toll-like receptor 4 (TLR4), Transforming growth factor (TGF), Tumor necrosis factor alpha (TNFα), Vascular endothelial growth factor (VEGF), Vitamin D receptor (VDR).*

TABLE 2B
MAJOR ALLELES OF HLA COMPLEX[#] (6P21-22) ASSOCIATED WITH SARCOIDOSIS

HLA class	HLA gene	Chromosomal location	Risk Alleles	Possible Association	References
Class I	HLA-A	29,909,037 - 29,913,661 bp	A*1	Susceptibility	[14]
Class I	HLA-B	31,321,649 - 31,324,965 bp	B*8	Susceptibility	[111-113]
Calss II	HLA-DPB1	33,043,703 - 33,054,978 bp	*0402	Protective	[57]
Class II	HLA-DQB1	32,627,244 - 32,636,160 bp	*0201	Protection, LS, mild disease, good prognosis	[114, 115]
			*0501	Protection	[116]
			*0602	Susceptibility/disease progression	[114, 115, 117]
Class II	HLA-DRB1	32,546,546 - 32,557,625 bp	*01, *04	Protection	[14, 67, 116]
			*0301	Acute onset/good prognosis, LS	[55, 67, 112, 113, 116, 118]
			*1101	Susceptibility	[119]
			*12	Significant with chronic disease	[116]
			*1401	Significant with chronic disease	[116, 118]
			*1501	Susceptibility	[67, 118, 119]
Class II	HLA-DRB3	32,470,408 - 32,484,153 bp	*0101	Susceptibility/disease progression	[120, 121]
Haplotype: HLA-DRB1*0401-DPB1*0401				Resolved sarcoidosis	[57]
Haplotype: HLA-DRB1*1501-DQB1*0602				Strong positive marker for severe pulmonary sarcoidosis	[122]
Haplotype: HLA-DRB1*04-DQB1*0301				Risk factor for disease manifestation but overall protective for sarcoidosis	[116]
Haplotype: HLA-DRB1*0301-DRB3*0101				Pulmonary sarcoidosis in Scandinavian patients	[123]
Haplotype: HLA-DRB1*0301-DQB1*0201				Extensively reproduced with Löfgren's syndrome	[20]

[#] Figure 1- for chromosomal localization

TABLE 3
FINE MAPPING OF SUSCEPTIBILITY LOCI FOR SARCOIDOSIS**

Loci/ microsatellite marker	Population studied	Clinical significance	References
2p25, 5q35, 9q34, 11p15 and 20q13	African Americans	Candidate regions for sarcoidosis	[62, 63]
5p15.2	African Americans	Gene protective for sarcoidosis	[63]
5q11.2	African Americans	Sarcoidosis susceptibility	[62, 63]
1p22 (D1S1665), 3p21 (D3S1766), 6p21-22 (*D6S1666* in MHC Class III), 7q22 (D7S821), 7q36 (D7S3070), 9q33 (D9S934), and X chromosome (DXS6789).	German families	Locus heterogeneity of susceptibility to sarcoidosis, with a major effect of the MHC	[61]

Microsatellite markers are given in parenthesis. **Figure 1- for chromosomal localization

D. MHC related genes as genetic risk factor of Sarcoidosis

Located in close proximity within the MHC region, the non-HLA class III comprises of genes such as, *tumor necrosis factor-alpha* (*TNFα*), *butyrophilin like 2* (*BTNL2*) and genes for complement protein, *C4A* and *C4B* that reside between the HLA class I and II genes.

The major proinflammatory cytokine, *TNFα*, has been implicated as sarcoidosis candidate gene across many populations in numerous studies [46-52]. Its role has been also been confirmed in a meta-analysis [52]. The polymorphism in promoter region of *TNFα* (rs1800629, -308G/A), which is in linkage with HLA-B8/DR3 [53] was widely investigated and *TNFα* -308 A allele was predominantly associated with LS in several studies [46-52]. Besides, *lymphotoxin-A* (*LTA*; rs909253, +252A/G) was reported to be associated with LS in Czech patients [49] and with erythema nodosum in female Caucasian [54] and in Polish patients [46] as a specific manifestation of sarcoidosis. Also, a strong LD among alleles *TNFα* -308A, *LTA* +252G and HLA-DRB1*03 has been associated with LS in Czech patients [49].

A close association among *BTNL2* and its proximally located HLA class II alleles has been reported with sarcoidosis in several studies [55-57]. *BTNL2* rs2076530(A) allele in sarcoidosis was observed in linkage disequilibrium (LD) with HLA-DRB1 alleles in the subgroup of patients with LS among Portuguese cohort of patients [55]. Among Japanese patients, HLA-DRB1*0803 (P = 6.15 x 10-5, OR = 2.43) and rs2076530(A) was associated (P = 6.9 x 10-6, OR = 1.84) with disease susceptibility suggesting HLA-DRB1 allele as major contributing genetic factor in the development of sarcoidosis in Japan [56]. In Finnish population, certain *BTNL2*-HLA-DRB1-DPB1 haplotypes were described with either predisposing [rs2076530(A)-DRB1*03:01-DPB1*01:01] or protective [rs2076530(G)-DRB1*01:01-DPB1*04:02] associations for sarcoidosis, corroborating the previous allele associations. Conversely, the same rs2076530(G) allele was found both as susceptible (e.g. rs2076530(G)-DRB1*04:01-DPB1*04:01), as well as protective haplotypes (e.g. rs2076530(G)-DRB1*01:01-DPB1*04:02) [57].

E. Differentially expressed genes in sarcoidosis for identification of disease specific gene variants

Recently, gene expression analysis has been used to identify networks of candidate genes involved in sarcoidosis [58,59]. A comparative gene array study on lung tissue from healthy controls and sarcoidosis patients identified higher expression of gene networks involving Th1 type antigen responses (IL-7, IL-15, the transcription factor family *STAT1*, and lymphocyte chemoattractant genes) and proteases *MMP-12* as well as *ADAMDEC1* [58]. Similarly, several differentially expressed genes were identified in patients with progressive pulmonary sarcoidosis [59]. In a comprehensive study, genome-wide peripheral blood gene expression analysis in a cohort of sarcoidosis patients identified 20 genes as biomarker signature for complicated sarcoidosis [60]. They also validated significant association of single nucleotide polymorphisms (SNPs) in signature genes with sarcoidosis susceptibility and severity.

F. Fine mapping of loci susceptibility for sarcoidosis

Genomic coordinates for location of variants / loci associated with sarcoidosis indicate the short arm of chromosome 6 (6p21-22) as promising region having several candidate genes as well as rich array of HLA alleles (Table 2 a,b). However, other regions including non-functional loci / co-dominant markers may also be important as possible genetic biomarkers for the disease. Genome-wide search for sarcoidosis susceptibility loci based on 225 microsatellite markers in German families showed most prominent marker at 6p21-22 (D6S1666) in MHC class-III region (Table 3). Six minor markers at regions 1p22 (D1S1665), 3p21 (D3S1766), 7q22 (D7S821), 7q36 (D7S3070), 9q33 (D9S934), and on long arm of X chromosome (DXS6789) were also identified [61]. The study suggested locus heterogeneity as susceptibility to sarcoidosis. From another genome wide approach study in African-Americans, D5S2500 on region 5q11.2 was the most prominent peak identified along with several linkage peaks on chromosome 5 [62]. The author urged for fine mapping of these linked regions, and in continuation to these findings, Gray-McGuire et al. reported D5S407 on 5q11.2 as strongest marker for the disease and 5p15.2 region with protecting role [63]. Combining the findings of Schurmann et al. (2005) and Gray-McGuire (2006), regions 2p25, 5q35, 9q34, 11p15 and 20q13 were in common indicating them as more reliable marker.

G. Possible contribution of Copy Number Variations to pathogenesis of sarcoidosis

Frequent changes in copy number variations (CNVs) may affect up to 12% of individual genome with functional alteration in nearby genes and, therefore, these could be among the candidates for association with complex disorders [64]. However, till now, studies that have been made towards CNVs in sarcoidosis susceptibility are very infrequent. Earlier, due to its possible involvement in sarcoidosis and being in proximity to *BTNL2* gene, CNV-507 (CNV_ID 507) was highlighted among 1447 copy number variable regions (CNVRs) detected in four populations with ancestry in Europe, Africa or Asia [65]. In contrast, in an another study, CNV_ID 507 was reported to have no effect on the genomic organization of *BTNL2* analyzing a cohort of 89 sarcoidosis patients and 89 matched controls [64]. Deficiencies of the fourth component of human complement C4 isotypes, *C4A* and *C4B* have been associated with various autoimmune, inflammatory or infectious diseases [66, 67]. Most Caucasians have 2 copies of *C4A* and *C4B* genes and low CNV (less than 2 copies) and *C4A* silencing (due to CT-insertion in codon 1213) made the *C4* as potential candidate gene for sarcoidosis. In Finnish sarcoidosis patients, *C4A* deficiency was associated with the disease, but *C4A* and *C4B* CNV or deficiencies did not differ among resolved and persistent patient groups [67]. Further studies are, therefore,

needed for exploring the potentiality of CNVs in disease susceptibility/ protection.

III. PERSPECTIVES AND FUTURE DIRECTIONS

As there is no well established animal model for sarcoidosis to date, we need to rely on information from studies in humans, either genetic or *ex-vivo* experiments. With limitation of information available in European cohorts only [68] it is necessary to perform meta-analysis of GWASs in large cohorts across the world populations such as American (including Hispanics), African and Japanese for discovering genetic risk variants associated with the disease prognosis and organ involvement. Further, GWAS studies and fine mapping of susceptibility loci for sarcoidosis indicate most of the variants to be located outside the coding sequence, therefore, along with focus on non-synonymous changes, it would be ideal to pool the information from variations at the non-coding regions/ approaches. These include identification of variations in the promoter/ regulatory sequences of candidate genes affecting binding sites for important putative transcription factors (TFs), epigenetic changes (such as, DNA methylation, histone modification and chromatin remodeling), exploring more CNVs, rare variants and their association with the disease. Also, immune response is modulated by the action of microRNAs (miRNA) which primarily targets 3'-untranslated region (UTR) of gene transcripts involved in the disease. For e.g., *miR-145* that binds to 3'-UTR of *OS9* is highly expressed (2.8 fold) in blood of sarcoidosis patients compared to healthy controls ($p=3.9 \times 10^{-3}$) [69] and is reported to be involved in airway inflammation in mice [70]. There are polymorphisms also within miRNA genes, so called mirSNPs, which bring yet another level of complexity.

Finally, it is apparent that sarcoidosis is one of many immune disorders mediated inflammatory disease with a complex etiology and is associated with the polygenic and environmental factors. The GWASs in future should focus on meta-analysis, "deep" disease phenotyping involving different ethnic groups for better understanding the mechanism of sarcoidosis immunopathogenesis .

IV. CONCLUSIONS

Immunogenetic and other linked genetic studies of sarcoidosis have brought along a number of genes, some of which, such as *HLA* gene cluster, *TNFA*, *BTNL-2* loci have been consistently replicated, other variants, e.g. *ANXA11* have been recently posed by GWAS results. However, before we may utilize some of these findings for prognostication of sarcoidosis clinical course and/or for assessment of therapeutic response, we need to have data available from further studies to address clinical utility of the nominated genetic markers and/or their combinations.

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