Chapter 3

Genetic Factors Involved in Sarcoidosis

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1. Introduction

Sarcoidosis is an immune mediated disease thought to be caused by complex interaction between genetic and environmental factors. Involvement of genetic factors in sarcoidosis is supported by familial clustering, increased concordance in monozygotic twins and varying incidence and disease presentation among different ethnic groups. Studies have revealed several human leukocyte antigen (HLA) and non-HLA alleles consistently associated with sarcoidosis susceptibility. Two genome scans have been reported in sarcoidosis: one in African Americans reporting linkage to chromosome 5 and the other in German families reporting linkage to chromosome 6. Follow-up studies on chromosome 6 identified the BTNL2 gene, a B7 family costimulatory molecule to be associated with sarcoidosis. Recent genome-wide association studies have found annexin A11 and RAB23 genes associated with sarcoidosis. The ongoing refinement of genetic marker maps, genotyping technology, and statistical analyses makes genomic exploration for sarcoidosis genes appealing.

2. Evidence for genetic predisposition to sarcoidosis

Familial sarcoidosis was first noted in Germany in 1923 by Martenstein, who reported two affected sisters [1]. After that several familial cases were reported across Europe and USA. Worldwide surveys revealed that familial sarcoidosis occurred in 10.3% cases from the Netherlands [2], 7.5% from Germany [3], 5.9% from the United Kingdom [4], 4.7% from Finland [5], 4.3% from Japan [5], 9.6% from Ireland[6] and 6.9 % from Sweden[7]. A family history survey of Detroit clinic–based population in USA showed that 17% of African Americans and 3.8% of white American reported a family history in first- and second degree relatives[8]. In African Americans, the sibling recurrence risk ratio, which compares disease risk among

siblings with the disease prevalence in the general population, is about 2.2 (95% confidence interval [CI], 1.03–3.68) [9].

The main limitation of these familial reports is the lack of a comparison group, and therefore it was unclear whether variation in familial sarcoidosis is due to variation in familial aggregation of disease risk, disease prevalence, or both. This question was addressed in the multicenter Case-Control Etiologic Study of Sarcoidosis (ACCESS) which evaluated 706 cases and matched controls [10]. It showed that the siblings of the affected patients had the highest relative risk (odds ratio =5.8 and 95% confidence interval=2.1–15.9). The odds ratio for the parents was 3.8 (95% CI=1.2–11.3) [10]. White cases had a markedly higher familial relative risk compared with African-American cases (18.0 versus 2.8; p=0.098).

A registry-based twin study in the Danish and the Finnish population showed an 80-fold increased risk of developing sarcoidosis in monozygotic co-twins and 7-fold increased risk in dizygotic twins [11].

Differences in disease incidence among different ethnic and racial groups exist worldwide. In the United States, African Americans have about a threefold higher age-adjusted annual incidence; 35.5 per 100,000 compared with Caucasians, 10.9 per 100,000. African American females aged 30 to 39 years were found at greatest risk at 107/100,000.The lifetime risk was calculated to be 2.4% for African Americans and 0.85% for Caucasian Americans [12]. In the United Kingdom, prevalence of sarcoidosis was found to be three times higher in the Irish living in London than in native Londoners [14]. It was eight time more common in natives of Martinique living in France than in the indigenous French populations [14]. In London the annual incidence of sarcoidosis has been reported as 1.5 per 100, 000 for Caucasians, 16.8 per 100, 000 for Asians and 19.8 per 100, 000 for Africans [15]. A study of a Swedish urban population reported a lifetime risk of 1.0% and 1.3% for men and women, respectively [16]. In addition to differences in the incidence, the clinical presentation of sarcoidosis also shows characteristic variability between ethnic groups. In both Blacks and Asians the disease has been reported to be more common, more severe and more extensive than in Caucasians [13, 15].

3. Genetics of other granulomatous disease

Blau syndrome and Crohn's disease

Among the granulomatous diseases with a putative genetic component, perhaps the most intriguing are Blau syndrome and Crohn's disease. Blau syndrome is an autosomal dominant granulomatous disease which is characterized by an early onset (before age 20) and involvement of skin, eye, and joints, similar to sarcoidosis. The factors that distinguish Blau syndrome from sarcoidosis are a lack of pulmonary involvement and absence of Kveim reactivity [17]. Crohn's disease is a familial granulomatous inflammatory bowel disease which, like sarcoidosis, may present with uveitis, arthritis and skin rash. Crohn's disease may involve the lung however the pattern of lung involvement differs from sarcoidosis.

Mutation in CARD (caspase activating recruitment domain) 15 gene, located on chromosome 16, is responsible for Blau syndrome [17, 18] and Crohn's disease [19]. Nucleotide oligomerization domain protein-2 (NOD2), encoded by CARD15, recognizes peptidoglycan, a component of bacterial cell walls, and is expressed mainly by antigen-presenting cells and epithelial cells [20]. Activation of NOD2 leads to nuclear factor (NF)-κB activation [20]. Rybicki and colleagues tested 35 African American affected sib pairs by using exclusion mapping and showed that the Blau syndrome/IBD1 locus did not confer risk for sarcoidosis [21]. Schurmann and coworkers [22] evaluated four main coding CARD15 polymorphisms associated with increased risk of Crohn's disease in both case–control and family-based sarcoidosis samples and concluded that CARD15 mutations play no role in sarcoidosis. Kanazawa and colleagues using a small sample analyzed 10 patients with early-onset sarcoidosis who had disease onset ranging from 6 months to 4 year of age and found that 9 of the 10 cases had heterozygous missense mutations in the CARD15 gene [23]. In conclusion, while an attractive candidate, no firm evidence exists to support a role for CARD 15 in sarcoidosis risk.

Chronic beryllium disease

Chronic beryllium disease (CBD), a chronic granulomatous lung disease caused by exposure to beryllium, shares similar histological and clinical findings with sarcoidsois. Glu69, carried by allele HLADPB1* 0201, was found not to be associated with sarcoidosis [24, 25]. In a study of 33 cases and 44 exposed persons without CBD (controls), Richeldi and colleagues found Glu69 in 97% of cases and in 30% of control subjects [26]. This HLA-DPB1 Glu69 association in beryllium disease has been widely supported [27] but is not associated with sarcoidosis.

Tuberculosis and leprosy

Polymorphic variants of the natural resistance–associated macrophage protein-1 gene (NRAMP1), now named SLC11A1, have been found to be associated with tuberculosis and leprosy susceptibility in endemic areas of disease [28, 29]. SLC11A1 is expressed primarily in macrophages and polymorphonuclear leukocytes and immunolocalization studies demonstrate the presence of NRAMP1 in lysosomes [30]. SLC11A1, an attractive candidate, was found not to increase the risk of sarcoidosis among African Americans [31], although a more recent article has noted an association in Polish patients (OR, 1.68; 95% CI, 1.01–2.81) [32].

4. Genetic associatiation studies in sarcoidosis

Genetic studies in sarcoidosis have gone through three phases – candidate gene studies, genome scanning using affected sib pair (ASP) linkage analysis and most recently, genome wide association studies (GWAS).

4.1. Candidate gene approach

The search for sarcoidosis susceptibility genes has generally relied on the candidate gene approach [33]. Investigators have selected genes for study that fit into the prevailing disease model. Sarcoidosis is thought to be a dysregulated response to an inhaled antigen that involves

antigen-presenting cells, T cells (primarily a helper T-cell type 1 polar response), and cytokine and chemokine release resulting in cell recruitment and the formation of granulomas in involved organs.

4.1.1. Association with Human Leukocyte Antigens (HLA)

HLA genes have been the best studied candidate genes in sarcoidosis. HLA genes are involved in presenting antigen to T cells and are grouped into three classes: class I, II and III. HLA association studies in sarcoidosis began over thirty years ago. A summary of the most consistent HLA associations in sarcoidosis is shown in Table 1. In 1977 Brewerton and colleague [34] first revealed an association of acute sarcoidosis with the HLA class I antigen HLA-B8 which was later confirmed by other groups [35, 36]. Hedfors and co-workers [35] also noted that HLA-B8/DR3 genes were inherited as a sarcoidosis risk haplotype in whites. In white HLA-B8/DR3 haplotype is associated with wide variety of autoimmune diseases [37]. These earlier studies of class I HLA antigens directed to the studies focused on HLA class II. A recent report by Grunewald and colleagues [38] suggests that HLA class I and II genes work together in sarcoidosis pathophysiology.

HLA gene	HLA class	Chromosome location	Risk Alleles	Putative Functional Significance
HLA-A	Class I	30,018, 309-30, 021, 041 bp	A*1	Susceptibility
HLA-B	Class I	31, 431, 922-31, 432, 914 bp	B*8	Susceptibility in several populations
HLA-DQB1	Class II	32, 735, 918-32, 742, 420 bp	*0201 *0602	Protection, Lofgren's syndrome, mild disease in several populations Susceptibility/disease progression in several groups
HLA-DRB1	Class II	32, 654, 526-32, 665, 559 bp	*0301 *01, *04 *1101	Acute onset/good prognosis in several groups Protection in several populations Susceptibility in whites and African Americans. Stage II/III chest X-ray
HLA-DRB3	Class II	32, 654, 526-32, 665, 540 bp	*1501 *0101	Associated with Lofgren's syndrome Susceptibility/disease progression in whites
BTNL2	Class II	32, 470, 490-32, 482, 878 bp	rs2076530	*BTNL2* rs2076530 G → A is associated with sarcoidosis risk in white patients but not in black patients.

Table 1. Summary of the most consistent HLA association studies in Sarcoidosis.

Among the HLA class II antigens, HLA-DRB1 have been the most studied antigen associated with sarcoidosis. The variation in the HLA-DRB1 gene affects both susceptibility and prognosis in sarcoidosis [39, 40]. In the ACCESS study, the HLA-DRB1* 1101 allele was associated with sarcoidosis both in blacks and whites ($p<0.01$) and had a population attributable risk of 16% in blacks and 9% in whites [41]. In addition susceptibility markers, the ACCESS study also found that HLA class II alleles might be markers for different phenotypes of sarcoidosis such as RB1*0401 for eye involvement in blacks and whites, DRB3 for bone marrow involvement in blacks, and DPB1*0101 for hypercalcemia in whites [41]. Another consistent finding across populations has been the HLA-DQB1*0201 allele association with decreased risk and lack of disease progression [42]. Other reports strongly support the notion that several different HLA class II genes acting either in concert or independently predispose to sarcoidosis [42-44]. Linkage disequilibrium (LD) within the major histocompatibility complex (MHC) region limits the ability to precisely identify the involved HLA genes. LD exists when alleles at two distinctive loci occur in gametes more frequently than expected. Grunewald and colleagues showed that the HLA-DRB1*03 associated with resolved disease and HLA-DRB1*15 with persistent disease were synonymous with HLA-DQB1*0201 with resolved disease and HLA DQB1*0602 with persistent disease [38]. Consequently, determining the effects of HLA-DQB1 on sarcoidosis risk apart from DRB1 or dissecting out other gene effects from closely linked haplotypes in the MHC region may be an intractable problem in whites. In African Americans, HLA-DRB1/DQB1 LD may not be as strong as in Caucasians [45].

HLA alleles have been consistently associated with disease course which suggests that HLA may play greater role in determining phenotype. Furthermore, the discrepant findings in HLA association among susceptibility studies could be explained by the phenotype variation in composition of the sarcoidosis patient groups studied.

4.1.2. Association with Non-HLA candidate genes

Genes that influence antigen processing, antigen presentation, macrophage and T-cell activation, and cell recruitment and injury repair may be considered sarcoidosis candidate genes. A summary of non-HLA candidate genes reported to date is shown in Table 2.

Angiotensin-Converting Enzyme

Angiotensin-converting enzyme (ACE) is produced by sarcoidal granulomas and its serum level can be elevated in sarcoidosis. Serum ACE levels are thought to reflect granuloma burden. The ACE gene insertion (I)/deletion (D) polymorphism partially accounts for the serum ACE level variation, and investigators have proposed that this genotype should be used to adjust serum ACE reference values [46]. Studies to support a role for ACE gene polymorphisms in susceptibility or severity have been inconsistent. While only a few case control studies have suggested that ACE gene polymorphism is associated with sarcoidosis susceptibility and disease severity [47, 48], most of the studies does not support that findings [50-53].

Candidate Gene	Chromosome Location	Association*†	Putative Functional Significance
Angiotensin-converting enzyme (ACE)	17q23	C	Increased risk for ID and DD genotypes. Moderate association between II genotype and radiographic progression.
C-C chemokine receptor 2	3p21.3	C+/-	Protection/Lofgren's syndrome association
C-C chemokine receptor 5	3p21.3	C-	Association of CCR5Delta32 allele more common in patients needing corticosteroid therapy. Refuted with haplotype analysis and larger sample.
Clara cell 10 kD protein	11q 12-13	C	An allele associated with sarcoidosis and with progressive disease at 3 year follow-up.
Complement receptor 1	1q32	A	The GG genotype for the Pro1827Arg (C (5,507) G) polymorphism was significantly associated with sarcoidosis.
Cystic fibrosis trans-membrane regulator	7q31.2	A+/-	R75Q increases risk.
HSPA1L heat shock protein 70 1 like	6p21.3	c	HSP(+2437)CC associated with susceptibility and LS
Inhibitor kβ-α	14q13	C	Association with -297T allele. Association of haplotype GTT at -881, -826, and -297, respectively. Allele -827T in Stage II.
Interleukin -1α	2q14	A	The IL-1α -889 1.1 genotype increased risk.
Interleukin -4 receptor	16p11.2		No association detected in 241 members of 62 families
Interleukin -18	11q22	A+/-	Genotype -607CA increased risk over AA. No association with organ involvement.
Interferon-γ	9p22	A	IFNA17 polymorphism (551T→G) and IFNA10 (60A) IFN-α 17 (551G) haplotype increased risk.
Toll-like receptor (TLR) 4 TLR10-TLR1-TLR6 cluster	9q32 4	B	Asp299Gly and Thre399Ile mutations associated with chronic disease Genetic variation in this cluster is associated with increased risk of chronic disease
Transforming growth factor (TGF)	19q13.2	B	TGF-β2 59941 allele, TGF-β3 4875 A and 17369 C alleles were associated with chest X-ray detection of fibrosis.
Tumor necrosis factor (TNF-α)	6p21.3	C+/-	Genotype -307A allele associated with Lofgren's syndrome and erythema nodosum and -857T allele with sarcoidosis. -307A not associated in African Americans.
Vascular endothelial growth factor(VEGF)	6p12	C	Protective effect of +813 CT and TT genotypes. Lower FEV1/FVC ratio observed with -627 GG genotype.
Vitamin D receptor	12q12-14	A-	B allele elevated in sarcoidosis patients

* Type of association: A = susceptibility; B = disease course; C = both.

† Association replicated (+); association refuted (-)

Table 2. A summary of Non-HLA candidate gene associated with Sarcoidosis

CC-Chemokine Receptor 2 (CCR2]

CCR 2, a receptor for monocyte chemoattractant protein, plays an important role in recruiting monocytes, T-cells, natural killer cells and dendritic cells [54]. CCR2 knockout mice die rapidly when challenged with mycobacteria [55] and display decreased IFN-γ production when challenged with *Leishmania donovani* or *Cryptococcus neoformans* [56, 57]. A single nucleotide polymorphism (SNP) in CCR2 gene (G190A, Val64Ile) is associated with protection in Japanese patients [58]. Evaluation of eight SNPs in the CCR2 gene in 304 Dutch patients showed that haplotype 2 was associated with Lofgren's syndrome [59]. Underrepresentation of the Val64Ile variant was observed in 65 Czech patients and in 80 control subjects but did not achieve statistical significance [60]. Despite using case control–based and family-based study designs and a sample much larger than the previous three studies, Valentonyte and colleagues could not replicate the CCR2 association [61].

C-C chemokine Receptor 5 (CCR5)

CCR5 serves as a receptor for CCL3 (macrophage inflammatory protein 1-α), CCL4 (macrophage inflammatory protein 1-β), CCL5 (RANTES [regulated upon activation, T-cell expressed and secreted]), and CCL8 (monocyte chemotactic protein 2) [62, 63]. A 32 bp deletion in the CCR5 gene results in a non-functional receptor unable to bind its ligands [64]. Petrek and colleagues reported that 32-bp deletion in CCR5 gene was significantly increased in Czech patients [60], whereas Spagnolo and colleagues, using haplotype analysis, found no association in evaluating 106 white British patients and 142 control subjects and 112 Dutch patients and 169 control subjects [65].

Clara cell 10 kD protein gene

Clara cells act as stem cells in bronchial epithelial repair, provides xenobiotic metabolism, and counter regulates inflammation [66]. Clara cell 10-kD protein (CC10) has been shown to inhibit IFN-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β. Murine and human CC10 gene promoter regions contain sites where inflammatory mediators, such as TNF-αand INF-α, -β, and –γ, alter transcriptional activity [67]. Increased level of CC10 in serum and BAL has been found in sarcoidosis patients whose disease had resolved compared with those whose disease had progressed [68]. The CC10 gene consists of three short exons separated by a long first and short second intron. An adenine to guanine substitution at position 38 (A38G) downstream from the transcription initiation site within the noncoding region of exon 1 has been the most studied CC10 polymorphism. The A/A genotype is believed to result in decreased CC10 levels [69]. The CC10A allele was found to be associated with sarcoidosis by Ohchi and colleague [70]. However association with the CC10 A38G polymorphism was not replicated in Dutch population or in Japanese subjects by Janssen and colleagues [71].

Complement receptor 1

Complement receptor 1 (CR1; CD35) is present on polymorphonuclear leukocytes, macrophages, B lymphocytes, some T lymphocytes, dendritic cells, and erythrocytes [71]. Immune complexes bound to CR1 are transferred to phagocytes as erythrocytes traverse the liver and spleen [72]. Immune complex clearance rates correlate with CR1 density. Low expression of

erythrocyte CR1 is associated with impaired immune complex clearance and deposition outside the reticuloendothelial system [73]. These extrareticuloendothelial immune complex deposits incite local inflammatory responses and presumably granuloma formation. That immune complexes may be involved in sarcoidosis was suggested in the early 1970s. In a series involving 3,676 patients from 11 cities around the world, James and coworkers [74] reported elevated serum γ-globulin levels above 3.5 g/100 ml in 23 to 96% of patients, with IgG being the most consistently and persistently elevated [75]. The different sensitivities of the techniques used explain in part the wide range in γ-globulin levels. It is generally accepted that immune complexes are always present in sarcoidosis depending on when and how they are detected. Zorzetto and colleagues have been the only group to report a CR1 gene association with sarcoidosis [76]. The GG genotype for the Pro1827Arg (C507G) polymorphism was associated with sarcoidosis versus healthy control subjects (odds ratio [OR), 3.13; 95% CI, 1.49–6.69) and versus control subjects with chronic obstructive pulmonary disease (OR, 2.82; 95% CI, 1.27–6.39). The GG genotype was most strongly associated with disease in female patients (OR, 7.05; 95% CI, 3.10–1.61) versus healthy control subjects. No relationship with clinical variables was found.

Cystic fibrosis transmembrane conductance regulator

The R75Q mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) occurs in high frequency in patients with atypical mild cystic fibrosis [77], bronchiectasis, and allergic bronchopulmonary aspergillosis [78]. Bombieri and colleagues reported a R75Q association with sarcoidosis [79], but in followup using complete cystic fibrosis gene mutation screening they could not replicate their findings [80]. Schurmann and colleagues could not demonstrate a CFTR association with sarcoidosis [81].

Heat shock protein A1L

Heat shock proteins (HSPs) comprise a conserved group of proteins with an average weight of 70 kD. Intracellular HSPs serve as molecular chaperones [82], whereas extracellular HSPs induce cellular immune responses [83]. HSPs may also act as carrier molecules for the immunogenic peptides presented on antigen-presenting cells [84]. Polymorphisms in the HSPA1L (alias HSP70-hom) have been associated with susceptibility to rheumatoid arthritis [85]. Antibodies to HSP70 in sarcoidosis have been reported [86, 87]. To further evaluate the role of HSPs in sarcoidosis, the HSP70 +2437 C allele was evaluated and found to be associated with sarcoidosis and Lo° fgren's syndrome in Polish patients [88] but not in Japanese patients [89].

Inhibitor κB-*α*

Inhibitor κB (IκB) masks the nuclear factor (NF)- κB nuclear localization sequence, thus retaining NF-κB in the cytoplasm and preventing DNA binding. On phosphorylation, IκB degrades, allowing NF-kB's nuclear localization and initiation of transcription [90]. Terminating the NF-κB response requires IκB-*α*. IκB-*α* knockout mice die 7 to 10 days after birth with increased levels of TNF-*α* mRNA in the skin and severe dermatitis [91]. NF-κB–dependent signaling in alveolar macrophage makes NF-κB and thus IκB central to sarcoid pathophysiol-

ogy [92]. Abdallah and colleagues found the promoter -297T allele associated with sarcoidosis [93]. No other IκB studies in sarcoidosis have been reported.

Interlukin-1(IL-1)

IL-1β produced mainly by macrophages maintains T-cell alveolitis and granuloma formation. Hunninghake and colleagues also demonstrated higher IL-1β activity in the BALF of patients with sarcoidosis compared with normal subjects [94]. Mikuniya and colleagues suggested that the ratio of IL-1 receptor antagonist to IL-1β in sarcoidal alveolar macrophage culture supernatants could predict disease chronicity [95]. The IL-1α 5′ flanking –889 C allele was found nearly two times more commonly among Czech patients with sarcoidosis compared with control subjects [96].

Interleukin Receptor- 4 (IL-4R)

The inflammatory response in sarcoidosis is primarily Th1 mediated. IL-4 drives Th2 differentiation [97]. To test whether variation in the IL-4R gene confers susceptibility to sarcoidosis, Bohnert and colleagues typed 241 members of 62 families with 136 affected siblings and 304 healthy control subjects for three functional SNPs within the IL-4R gene and found no evidence for linkage or association, thus excluding a significant role for IL-4R [98].

Interlukin-18 (IL-18)

IL-18 produced by monocytes/macrophages induces IFN-γ and drives the Th1 response. BALF and serum IL-18 levels are increased in sarcoidosis [99]. An association between IL-18607 (A/C) polymorphism and sarcoidosis has been reported and refuted in Japanese [100, 101] and white subjects [102, 103].

Interferon–α (IFN-α)

The increasing number of reported cases of IFN-α–induced sarcoidosis supports that IFN-α is important in sarcoidosis [104]. Akahoshi and colleagues found an IFN-α T551G (Ile184Arg) polymorphism associated with sarcoidosis susceptibility (OR, 3.27; 95% CI, 1.44–7.46; p=0.004) [105]. This allele is also associated with high IFN-α production and subsequent strong Th1 polarization.

Transforming Growth Factor-β (TGF-β)

Polymorphisms for all three isoforms of transforming growth factor (TGF) – β (TGF- β1, TGF-β2, and TGF-β3) have been associated with protein expression variation or functionality changes [106]. TGF-β1 levels are increased in patients with sarcoidosis who have impaired pulmonary function [107]. Kruit and colleagues reported that the TGF-β2 59941Gallele and the TGF-β3 4875 A and 17369 C alleles were associated with chest X-ray evidence of pulmonary fibrosis [85]. The TFG-β3 15101 G allele was lower in patients with fibrosis [108].

Toll-like receptor 4 (TLR4) and TLR10-TLR1-TLR6 cluster

Toll-like receptor 4 (TLR4), the first and best described of the many TLRs, plays a crucial role in detecting infection and inducing inflammatory and adaptive immune responses [109]. Pabst and colleagues examined 141 white German patients and control subjects for the TLR4

polymorphisms Asp299Gly and Thre399Ile and found no association with disease presence but did find a significant correlation with chronic disease [110].

Recently Veltcamp and colleague found that genetic variation in TLR10-TLR1-TLR6 cluster is associated with increased risk of chronic disease [111].

Tumor Necrosis Factor–α (TNF-α)

TNF-α has a broad range of inflammatory and immunostimulatory actions, including orchestrating granuloma formation. TNF-α stimulates cytokine production, enhances expression of adhesion molecules, and acts as a costimulator of T-cell activation. Alveolar macrophages from patients with active sarcoidosis secrete more TNF-α than those with inactive disease [112]. TNF-α has been considered a target for therapy in sarcoidosis [113].

Although it is unclear whether TNF-α promoter polymorphisms are functionally significant, studies suggest that a small but significant effect of the TNF-α promoter -307 A/G polymorphism may exist, with the A allele being associated with slightly greater levels of TNF-α transcription [114, 115]. A higher frequency of TNF-307A allele has been found in patients presenting with Lofgren's syndrome and erythema nodosum [116–118]. In evaluating five promoter polymorphisms, Grutters and colleagues found a significant increase in TNF -857T allele in white British and Dutch patients and confirmed the TNF -307A allele association with Lo° fgren's syndrome [119]. In these studies, it is not clear whether TNF-307A confers independent risk from HLA-DRB1 because TNF is in tight LD with HLA-DRBI [120]. Using a family-based approach, TNF-α was not found to be significantly associated with sarcoidosis [49].

Vascular endothelial growth factor

Dysregulated vascular endothelial growth factor (VEGF) expression has been implicated in several inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases [121, 122]. VEGF modulates angiogenesis, enhances monocyte migration, a key event in granuloma formation [123]. Tolnay and colleagues reported increased VEGF transcription and protein production in activated alveolar macrophages in epithelioid cells and multinuclear giant cells of pulmonary sarcoidal granulomas [124]. Several polymorphisms have been associated with VEGF protein production [125, 126]. Morohashi and colleagues found that the VEGF+813T allele was underrepresented (associated with decreased risk) in patients with sarcoidosis. The +813 site is predicted to lie within a potential transcription factor binding site and could potentially reduce VEGF expression [126].

Vitamin D receptors

The active form of vitamin D, 1,25-dihydroxy vitamin D3, modulates the immune response through control of cytokine expression, including IFN-γ and IL-2 [127]. Increased expression of vitamin D receptors (VDRs) on sarcoidal BAL T cells and alveolar macrophage production of 1,25-dihydroxy vitamin D3 have been reported [128, 129]. Niimi and colleagues reported a VDR Bsm1 restriction site polymorphism in intron 8 to be associated with sarcoidosis [130]. Guleva and Seitzer examined a VDR Taq1 polymorphism in linkage disequilibrium with the BsmI polymorphism in 85 patients and 80 control subjects and could not confirm Niimi and

colleagues' findings [131]. Rybicki and colleagues also could not confirm VDRs as candidate genes in sarcoidosis [49].

CD80 and CD86

The B7 family of costimulatory molecules (CD80 and CD86) regulate T-cell activation. T-cell activation requires two signals: one mediated by T-cell receptor interaction with specific antigen in association with HLA molecules and an antigen-independent costimulatory signal provided by interaction between CD28 on T-cell surface and its ligands CD80 (B7-1) and CD86 (B7-2) on the antigen-presenting cells [146]. Handa and colleagues investigated CD80 and CD86 SNPs for sarcoidosis susceptibility in 146 Japanese patients and found no significant difference compared with 157 control subjects [147].

Unfortunately none of candidate gene chosen based on its likely function in sarcoidosis pathophysiology has been confirmed using the family-based study design. Limitation to many of these studies likely resides in the case-control study design's susceptibility to a form of confounding known as population stratification which can be overcome by using a family-based design that involves recruiting patients 'siblings and parents if available. In this design, parental alleles not transmitted to affected offspring are used as the control alleles and thus control for genetic background. The transmission disequilibrium test, one of the statistical methods used, counts the number of parental gene variants transmitted to affected offspring. Deviation from expected transmission supports a predisposing effect of the more frequently transmitted allele.

4.2. Genome scanning: Affected sib pair linkage analysis

Sarcoidosis genome scan in Germans

The first genome scan study related to sarcoidosis was conducted by Schurmann and colleagues, in which they used 225 microsatellite markers spanning the genome in 63 German families to identify a linkage signal (D6S1666) on chromosome 6p21 [132]. This group then used a three-stage single-nucleotide polymorphism (SNP) scan of the 16-MB region surrounding D6S1666 [133] and identified a single SNP, rs2076530, in the BTNL2 gene associated with sarcoidosis. This SNP (G/A) was found at the 3' boundary of the exon 5 coding region. The A allele at this position has been proposed to introduce an alternative splice site at the exon 5–3' intron boundary of the BTNL2 transcript that results in a premature truncation of the protein.

BTNL2, also known as "butyrophilin-like 2" and "BTL-2," is a butyrophilin gene that belongs to the immunoglobulin gene superfamily related to the B7 family [134, 135]. Butyrophilin was initially cloned from cattle mammary epithelial cells [136]. This gene was localized to the MHC class II region in humans. To determine the consistency of the BTNL2 gene as a sarcoidosis risk factor across different populations, Rybicki and colleagues characterized variation in the BTNL2 exon/intron 5 region in an African-American family sample that consisted of 219 nuclear families (686 individuals) and in 2 case–control samples (295 African-American matched pairs and 366 white American matched pairs) [137].They confirmed that BTNL2 somewhat was less associated with sarcoidosis in African Americans compared with whites. BTNL2 appears to have moderate influence on individual disease risk (odds ratio of 1.6 in

heterozygotes and 2.8 in homozygotes). The population attributable risk of 23% for heterozygotes and homozygotes indicates a significant contribution at the population level.

Whether BTNL2 as a sarcoidosis risk factor is independent of HLA-DRB risk alleles or not, still remains a question. HLADRB and BTNL2 are in linkage disequilibrium. Linkage disequilibrium is the nonrandom association of alleles physically closes on a chromosome. HLA-DRB lies about 180 kb centromeric to BTNL2. On the basis of regression models, BTNL2 appears to be an independent risk factor [133, 137]. In the case of blacks, in whom the BTNL2-conferred sarcoidosis risk is less significant than for whites, a negative interaction with HLA-DR appears to exist [137]. In one study, BTNL2 was found not to be associated with Wegener's granulomatosis [138].

Most recently Hofmann and colleagues [139] conducted a Genome-Wide Linkage Analysis in 181 German sarcoidosis families using clustered biallelic markers. This study revealed one region of suggestive linkage on chromosome 12p13.31 at 20 cM (LOD= 2.53; local *P* value =. 0003) and another linkage on 9q33.1 at 134 cM (LOD =2.12; local *P* value =.0009). It is proposed that these regions might harbor yet-unidentified, possibly subphenotype-specific risk factors for the disease (e.g. immune-related functions like the tumor necrosis factor receptor 1).

Sarcoidosis genome scan in African Americans

Eleven centers joined together in an NHLBI-sponsored effort (Sarcoidosis Genetic Analysis Consortium [SAGA]) to perform a genome scan in African American siblings. This group performed a 380-microsatellite genomewide scan across 22 autosomes in 519 African American sib pairs. The significant findings included 15 markers with p values < 0.05 with the strongest linkage signal on chromosome 5 [140]. Fine mapping studies indicated a sarcoidosis susceptibility gene on chromosome 5q11.2 and a gene protective effect for sarcoidosis on 5p15.2 [141].

The reason why African Americans were chosen to uncover sarcoidosis susceptibility genes was that African Americans are more commonly and severely affected and have affected family members more often than whites. But the disadvantage of doing so is that African Americans are admixed with white and other populations to varying degrees with possible admixture among their participating centers ranging from 12% in South Carolina to 20% in New York [142]. To address the possibility that admixed subpopulations existed in the SAGA sample and affected the power to detect linkage, the sample was stratified by genetically determined ancestry using the data from the 380 microsatellite markers genotyped in the genome scan. The African-American families were clustered into subpopulations based on ancestry similarity. Evidence of two genetically distinct groups was found: Stratified linkage results suggest that one subpopulation of families contributed to previously identified linkage signals at 1p22, 3p21-14, 11p15, and 17q21 and that a second subpopulation of families contributed to those found at 5p15-13 and 20q13 [143]. These findings support the presence of sarcoidosis susceptibility genes in regions previously identified but indicate that these genes are likely to be specific to ancestral groups that have combined to form modern-day African Americans.

4.3. Genome-Wide Association Studies (GWAS)

In genome-wide association study high throughput genotyping methods are used to genotype a dense set of SNPs across the genome. A significant advantage of this approach is that association studies are more powerful than affected sib pair methods of linkage analysis. Hofmann and colleagues [144] conducted a genomewide association study of 499 German patients with sarcoidosis and 490 control subjects. The strongest signal mapped to the annexin A11 gene on chromosome 10q22.3. Validation in an independent sample confirmed the association. Annexin A11 has regulatory functions in calcium signaling, cell division, vesicle trafficking, and apoptosis. Depletion or dysfunction of annexin A11 may affect the apoptosis pathway in sarcoidosis. Later the same group [145] reported another associated locus 6p12.1 that comprises several genes, a likely candidate being RAB23. RAB23 is proposed to be involved in antibacterial defense processes and regulation of the sonic hedgehog signaling pathway.

5. Counseling and screening

In the context of genetic family counseling, this generally is perceived as a small risk by the clients and should lead to enhanced awareness but does not justify specific medical investigations in the absence of complaints.

6. Genetic testing

Genetic testing at present does not play a role in the diagnosis and treatment of sarcoidosis.

7. Future directions

The cause of sarcoidosis remains unknown. It is thought to be caused by interaction between environmental and genetic factors. Genetic studies have revealed the HLA and other candidate genes associated with sarcoidosis susceptibility. Association studies have been motivated by the hopes that identifying alleles that affect risk and phenotype will help in understanding disease etiology. Unfortunately, many of the reported associations have not been replicated. Two genome scans have been reported and one has yielded a likely candidate gene, BTNL2 that has been replicated in large studies. Emerging technologies and advances in genomics and proteomics will help find the causes sarcoidosis, better understanding of pathogenesis of sarcoidosis and to test new therapy. Gene expression profiling in BALF and blood carried out at the time of presentation will likely help to better predict disease resolution or progression.

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