Genetics of Sarcoidosis
Candidate Genes and Genome Scans

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Human leukocyte antigen class II allele associations and T-cell receptor beta chain bias in sarcoidosis suggest a specific disease-triggering antigen exposure in a genetically susceptible host. The cause of sarcoidosis has been elusive, but genetics provides one of the few promising avenues to further our understanding. We review the association studies and genome scans used to identify the genes involved in sarcoidosis.

Keywords: sarcoidosis; genetics; genomics

Racial differences in incidence rates and disease clustering in families (1–3) support the belief that genetics contribute to sarcoidosis etiology. In the United States, African Americans are three and a half times more commonly affected (2), and incidence rates vary widely across ethnic groups in Europe and Asia. Worldwide, familial sarcoidosis occurs in 3 to 14% of patients. In the United States, African Americans are three times more likely than whites to have an affected first- or second-degree relative (4). 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) (3). The ACCESS study (A Case Control Etiologic Sarcoidosis Study) found that cases were five times more likely than control subjects to report an affected sibling or parent (1).

Investigators have relied mainly on candidate gene association studies to search for susceptibility genes in sarcoidosis. More recently, two genome scans, one in whites and the other in African Americans, have been reported. The ongoing refinement of genetic marker maps, genotyping technology, and statistical analyses makes genomic exploration for sarcoidosis genes appealing.

Because many sarcoidosis variants exist and because organ dysfunction may evolve over several years, it is often necessary to follow patients for several years to assign an accurate phenotype. Even patients who present with Löfgren's syndrome, which is a good prognostic sign, can develop chronic sarcoidosis many years later (5). A phenotyping scheme that may further aid linkage and association studies might be gained through evaluating clinical similarities among families with more than one member affected, but few studies exist. Valentonyte and colleagues, in a family analysis to evaluate the candidate gene CCR2, found that 25 of 75 informative sibling pairs exhibited discordance for acute or chronic sarcoidosis phenotypes, arguing against acute sarcoidosis as a distinct heritable phenotype (6). In a recent evaluation in 340 affected African-American sib pairs, Judson and colleagues found only a modest sib concordance for liver and ocular involvement despite evaluating 15 different organ systems (7).

(*Received in original form July 14, 2006; accepted in final form August 16, 2006*)

Supported by NIH grant HL60263 (M.C.I.) and by the Vivien Richenthal Research Institute, Mount Sinai School of Medicine.

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Proc Am Thorac Soc Vol 4. pp 108–116, 2007
DOI: 10.1513/pats.200607-141JG
Internet address: www.atsjournals.org

CANDIDATE GENE APPROACH

Investigators choose candidate genes that make sense based on the prevailing model of pathogenesis. Candidates have been chosen often based on their potential role in granuloma formation. Granuloma formation begins with the sarcoid antigen provoking mononuclear phagocyte and subsequent T-helper cell activation. Released chemokines and cytokines further regulate cell recruitment. Granuloma persistence and accumulation occur due to an inability to clear antigen. Perhaps sarcoidosis can be best thought of as "hereditary frustrated phagocytosis."

HUMAN LEUKOCYTE ANTIGENS

Given the importance of human leukocyte antigens (HLAs) in antigen presentation, it is not surprising that searching for HLA associations with sarcoidosis began over 30 yr ago. The first report revealed an association of acute sarcoidosis with the HLA class I antigen HLA-B8 (8). Other groups (9, 10) confirmed the HLA-B8 association and noted that HLA-B8/DR3 genes were inherited as a sarcoidosis risk haplotype (9), a haplotype also associated with autoimmune diseases in whites (11). These earlier studies of class I HLA antigens gave way to studies focused on HLA class II. A recent report by Grunewald and colleagues suggests that HLA class I and II genes work together in sarcoidosis pathophysiology (12). A summary of the most consistent HLA associations in sarcoidosis is shown in Table 1.

The HLA-DRB1 association predominates in the literature, with variation in the HLA-DRB1 gene affecting sarcoidosis susceptibility and prognosis (13, 14). Another consistent finding across populations has been the HLA-DQB1*0201 allele association with decreased risk and lack of disease progression (15). Other reports strongly support that several different HLA class II genes acting in concert or independently predispose to sarcoidosis (15–17). The 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. For example, 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 (12). 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, LD across the MHC region may not be strong (18).

A negative finding is the lack of association with HLA-DPB1*0201, the allele that carries glutamate in amino acid position 69 (Glu69) (16, 19). Glu69 has been associated with chronic beryllium disease, a disease similar to sarcoidosis histologically

TABLE 1. SUMMARY OF HLA ASSOCIATION STUDIES OF SARCOIDOSIS

HLA	Risk Alleles	Finding	Reference
HLA-A	A*1	Susceptibility	126
HLA-B	B*8	Susceptibility in several populations	8, 9, 127
HLA-DPB1	*0201	Not associated with sarcoidosis	16,19
HLA-DQB1	*0201	Protection, Löfgren's syndrome, mild disease in several populations	15, 128
	*0602	Susceptibility/disease progression in several groups	15, 129
HLA-DRB1	*0301	Acute onset/good prognosis in several groups	9, 127, 130
	*04	Protection in several populations	126
	*1101	Susceptibility in whites and African Americans. Stage II/III chest X-ray	13
HLA-DRB3	*1501	Associated with Löfgren's syndrome	13, 129
	*0101	Susceptibility/disease progression in whites	131

and clinically. In 33 berylliosis cases and in 44 exposed individuals without berylliosis, Richeldi and colleagues found Glu69 in 97% of cases and in 30% of control subjects (20). This HLA-DPB1 Glu69 association in beryllium disease has been widely supported (21) but is not associated with sarcoidosis.

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. Table 2 lists the non-HLA candidate genes reported to date.

Angiotensin-converting Enzyme

Epithelioid cells in granulomas produce angiotensin-converting enzyme (ACE), and serum angiotensin-converting enzyme (SACE) levels are believed to reflect granuloma burden. The ACE gene insertion (I)/deletion (D) polymorphism partially accounts for the SACE level variation, and investigators have proposed that this genotype should be used to adjust SACE reference values (22). Studies to support a role for ACE gene polymorphisms in susceptibility or severity have been inconsistent (23, 24).

CC-Chemokine Receptor 2

CC-chemokine receptor 2 (CCR2), a receptor for monocyte chemoattractant protein, plays an important role in monocyte/macrophage/lymphocyte trafficking (25). CCR2 knockout mice die rapidly when challenged with mycobacteria (26) and display decreased IFN-γ production when challenged with *Leishmania donovani* or *Cryptococcus neoformans* (27, 28). Investigators have reported that a single nucleotide polymorphism (C190A, Val64Ile) shows protection in Japanese patients (29) and that an eight–single nucleotide polymorphism haplotype is associated with Löfgren's syndrome in Dutch patients (30). Underrepresentation of the Val64Ile variant was observed in 65 Czech patients and in 80 control subjects but did not achieve statistical significance (31). 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 (6).

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) (32, 33). Despite CCR5 playing a dominant role in recruiting and activating lymphocytes and monocytes in sarcoidosis (34), no firm association with sarcoidosis susceptibility has been found. Petrek and colleagues reported an association of the 32-bp deletion in CCR5 in Czech patients (31), 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 (35).

CCR10

Clara cell 10-kD protein. Clara cells serve as a major respiratory tract protector, acting as stem cells in bronchial epithelial repair, providing xenobiotic metabolism, and counterregulating inflammation (36). 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-α and INF-α, -β, and -γ, alter transcriptional activity (37). Increased CC10 levels have been found in patients whose sarcoidosis had resolved compared with those whose sarcoidosis had progressed (38). The CC10 gene contains 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 results in decreased CC10 levels (39).

Ohchi and coworkers found that the CC10 A allele was associated with sarcoidosis (40). That patients with the A/A genotype had the lowest bronchoalveolar lavage fluid (BALF) levels of CC10 further strengthened this association. However, Janssen and colleagues found no association with the CC10 A38G SNP in a white Dutch population or in Japanese subjects (41).

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 (42). 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 (43).

Complement Receptor 1

Complement receptor 1 (CR1; CD35) is a widely distributed membrane glycoprotein present on polymorphonuclear leukocytes, macrophages, B lymphocytes, some T lymphocytes, dendritic cells, and erythrocytes (44). Immune complexes bound to CR1 are transferred to phagocytes as erythrocytes traverse the

TABLE 2. NON-HLA CANDIDATE GENE ASSOCIATIONS WITH SARCOIDOSIS

Candidate Gene	Location	Association*†	Comment	References
Angiotensin-converting enzyme	17q23	C	Increased risk for ID and DD genotypes Moderate association between II genotype and radiographic progression	23, 24, 132–134
C-C chemokine receptor 2	3p21.3	C+/−	Protection/LS association	6, 29–31
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.	31, 35
CD80, CD86	3q21		No association detected	43
Clara cell 10 kD protein	11q 12-13	C	A allele associated with sarcoidosis and with progressive disease at 3 yr follow-up.	40
Complement receptor 1	1q32	A	The GG genotype for the Pro1827Arg(C(5,507)G) polymorphism was significantly associated with sarcoidosis.	48
Cystic fibrosis transmembrane regulator	7q31.2	A+/−	R75Q increases risk.	51, 53
HSPA1L heat shock protein 70 1 like (alias heat shock protein 70-hom)	6p21.3	C	HSP(+2437)CC associated with susceptibility and LS	60
Inhibitor κB-α	14q13	C	Association with −297T allele. Association of haplotype GTT at −881, −826, and −297, respectively. Allele −827T in Stage II.	65
IL-1α	2q14	A	The IL-1α −889 1.1 genotype increased risk.	68
IL-4 receptor	16p11.2		No association detected in 241 members of 62 families	70
IL-18	11q22	A+/−	Genotype −607CA increased risk over AA. No association with organ involvement.	72–75
IFN-γ	9p22	A	IFNA17 polymorphism (551T→G) and IFNA10 [60A] IFN-α17 [551G] haplotype increased risk.	77
Natural resistance associated macrophage protein	2q35	A	Protective effect of (CA)(n) repeat in the immediate 5′ region of the NRAMP1 gene	81
Toll-like receptor 4	9q32	B	Asp299Gly and Thre399Ile mutations associated with chronic disease	87
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.	85
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.	23, 92–95
VEGF	6p12	C	Protective effect of +813 CT and TT genotypes	135
Vitamin D receptor	12q12-14	A−	BsmI allele elevated in sarcoidosis patients	23, 106, 107

Definition of abbreviations: IL = interleukin; LF = Löfgren's syndrome; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.
* Type of association: A = susceptibility; B = disease course; C = both.
† − = Association refuted; + = association replicated.

liver and spleen (45). 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 (46). 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 (47) 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 (47). 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 (48). 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 (49), bronchiectasis, and allergic bronchopulmonary aspergillosis (50). Bombieri and colleagues reported a R75Q association with sarcoidosis (51), but in follow-up using complete cystic fibrosis gene mutation screening they could not replicate their findings (52). Schurmann and colleagues could not demonstrate a CFTR association with sarcoidosis (53).

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 (54), whereas extracellular HSPs induce cellular immune responses (55). HSPs may also act as carrier molecules for the immunogenic peptides presented on antigen-presenting cells (56). Polymorphisms in the HSPA1L (alias HSP70-hom) have been associated with susceptibility to

rheumatoid arthritis (57). Antibodies to HSP70 in sarcoidosis have been reported (58, 59). To further evaluate the role of HSPs in sarcoidosis, the HSP70 +2437 C allele was evaluated and found to be associated with sarcoidosis and Löfgren's syndrome in Polish patients (60) but not in Japanese patients (61).

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-κB's nuclear localization and initiation of transcription (62). Terminating the NF-κB response requires IκB-α. IκB-α knockout mice die 7 to 10 d after birth with increased levels of TNF-α mRNA in the skin and severe dermatitis (63). NF-κB–dependent signaling in alveolar macrophage makes NF-κB and thus IκB central to sarcoid pathophysiology (64). Abdallah and colleagues found the promoter −297T allele associated with sarcoidosis (65). No other IκB studies in sarcoidosis have been reported.

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 (66). Mikuniya and colleagues have suggested that the ratio of IL-1 receptor antagonist to IL-1β in sarcoid alveolar macrophage culture supernatants could predict disease chronicity (67). The IL-1α 5′ flanking –889 C allele was found nearly two times more commonly among Czech patients with sarcoidosis compared with control subjects (68).

IL-4R

The inflammatory response in sarcoidosis is primarily Th1 mediated. IL-4 drives Th2 differentiation (69). 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 (70).

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 (71). An association between IL-18607 (A/C) polymorphism and sarcoidosis has been reported and refuted in Japanese (72, 73) and white subjects (74, 75).

IFN-α

The increasing number of reported cases of IFN-α–induced sarcoidosis supports that IFN-α is important in sarcoidosis (76). Akahoshi and colleagues found an IFN-α T551G (Ile184Arg) polymorphism associated with sarcoidosis susceptibility (OR, 3.27; 95% CI, 1.44–7.46; $p = 0.004$) (77). This allele is also associated with high IFN-α production and subsequent strong Th1 polarization.

SLC11A1

Polymorphic variants of the natural resistance-associated macrophage protein 1 gene, now named SLC11A1, have been associated with tuberculosis and leprosy susceptibility in endemic areas of disease (78, 79). Immunolocalization studies demonstrate the presence of SLC11A1 in lysosomes of macrophages and polymorphonuclear leukocytes (80). SLC11A1 was found not to increase the risk of sarcoidosis among African Americans (81), although a more recent article has noted an association in Polish patients (OR, 1.68; 95% CI, 1.01–2.81) (82).

Transforming Growth Factor-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 (83). TGF-β1 levels are increased in patients with sarcoidosis who have impaired pulmonary function (84). Kruit and colleagues reported that the TGF-β2 59941 G allele 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 (85).

Toll-like Receptor 4

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 (86). 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 (87).

TNF-α

TNF-α has a broad range of inflammatory and immunostimulatory actions, including playing a key role in 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 (88). TNF-α has been considered a target for therapy in sarcoidosis (89). 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 (90, 91). A higher frequency of TNF-307A allele has been found in patients presenting with Löfgren's syndrome and erythema nodosum (92–94). 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 Löfgren's syndrome (95). 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 (96). Using a family-based approach, TNF-α was not found to be significantly associated with sarcoidosis (23).

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 (97, 98). VEGF modulates angiogenesis and has been reported to enhance monocyte migration, a key event in granuloma formation (99). Tolnay and colleagues reported increased VEGF transcription and protein production in activated alveolar macrophages in epithelioid cells and multinuclear giant cells of pulmonary sarcoid granulomas (100). Several polymorphisms have been associated with VEGF protein production (101, 102). Morohashi and colleagues found that the VEGF+813T allele was underrepresented in patients with sarcoidosis (associated with decreased risk). The +813 site is predicted to lie within a potential transcription factor binding site and could possibly reduce VEGF expression (102).

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 (103). Increased expression of vitamin D receptors (VDRs) on sarcoid BAL T cells and sarcoid alveolar macrophage production of 1,25-dihydroxy vitamin D3 have been reported (104, 105). Niimi and colleagues reported a VDR Bsm1 restriction site polymorphism in intron 8 to be associated with sarcoidosis (106). 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 (107). Rybicki and colleagues also could not confirm VDRs as candidate genes in sarcoidosis (23).

Although the choice of each candidate gene (Table 2) makes sense based on their function, none have been consistently reproduced or confirmed in family studies. The variability in reported outcomes can be traced to methodologic differences between studies, such as the use of inappropriate control subjects (e.g., convenient samples, such as hospital personnel or patients with other diseases) or case heterogeneity. Furthermore, 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. If the gene under study shows marked variation in allele frequency across population subgroups and if these subgroups differ in their baseline disease risk, then spurious associations may arise. Because infection and immune surveillance appear under strong negative population pressure, immune response genes seem to be particularly subject to confounding by population stratification.

The issue of population stratification can be overcome. One way is to use 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. No candidate gene chosen based on its likely function in sarcoidosis pathophysiology has been confirmed using the family-based study design.

GENETIC ADVANCES IN OTHER GRANULOMATOUS DISEASE

Advances have been made in defining the genetics of inflammatory bowel disease and Blau syndrome, an autosomal dominant disease comprised of granulomatous uveitis, arthritis, and skin rash but without lung involvement or Kveim skin test positivity. CARD15 located on chromosome 16 is responsible for Blau's syndrome (108, 109) and is the gene identified in the inflammatory bowel disease 1 (IBD1) locus (110). NOD2, encoded by CARD15, recognizes peptidoglycan, a component of bacterial cell walls, and is expressed mainly by antigen-presenting cells and epithelial cells (111). NOD2 activation leads to NF-κB activation (111).

Using exclusion mapping among sarcoid-affected sib pairs, Rybicki and colleagues showed that the Blau syndrome/IBD1 locus did not confer risk for sarcoidosis (112). Further refuting CARD15 as a sarcoidosis susceptibility gene, Schurmann and colleagues, using case control–based and family-based samples, evaluated four coding CARD15 polymorphisms associated with increased risk of Crohn's disease and concluded that CARD15 mutations play no role in sarcoidosis susceptibility (113). Kanazawa and colleagues, however, analyzed 10 patients with early-onset sarcoidosis who had disease onset ranging from 6 mo to 4 yr of age (114). They reported that 9 of the 10 cases had heterozygous missense mutations in the CARD15 gene. Despite the negative studies reported by Rybicki and colleagues (112) and Schurmann and colleagues (113), the CARD15 mutations may play a role in the early-onset phenotype.

GENOME SCANNING: AFFECTED SIB PAIR LINKAGE ANALYSIS

Genomewide scans that use allele-sharing methods map disease genes by testing for marker similarity among affected relatives. If the affected individuals within a collection of pedigrees or sibships show an unusual propensity to share marker alleles identical by descent in a particular chromosomal region, then that region may harbor a disease gene. Genomewide scans offer the opportunity to detect candidate genes without regard to the current disease model but rather according to their chromosomal location detected by linkage signals.

First Sarcoidosis Genome Scan in Whites

Schurmann and colleagues, in the first sarcoidosis genome scan, used 225 microsatellite markers spanning the genome in 63 German families and found seven linkage peaks. The highest linkage signal was on chromosome 6p21 (D6S1666) (115). Despite the fact that this linkage signal represented only suggestive evidence of linkage with a nonparametric lod (logarithm of the odds) score of 2.99, the group used a three-stage SNP scan of the 16-MB region surrounding D6S1666 and identified a single SNP, rs2076530, in the BTNL2 gene associated with sarcoidosis (116). This SNP (G/A) was found at the 3′ boundary of the exon 5 coding region. The A allele at this position introduces an alternate splice site at the transcript's exon 5′-3′ intron that results in a premature truncation of the protein.

BTNL2 (aliases "butyrophilinlike 2" and "BTL-2") is a butyrophilin gene that belongs to the B7 family (42, 117). Butyrophilin was initially cloned from cattle mammary epithelial cells (118) and is a member of a family of genes located in the MHC class II region (117). Members of the butyrophilin gene family share gene architecture that consists of a signal peptide, two immunoglobulin domains (the first a larger variable domain and the second a smaller constant domain), a transmembrane domain and cytoplasmic region, heptad repeats, and a PKA domain at the carboxy terminus.

In evaluating the BTNL2 gene as a sarcoidosis risk factor in whites and African Americans, Rybicki and colleagues characterized the variation in the BTNL2 exon/intron 5 region in three samples that consisted of 219 nuclear African American families (686 individuals) and two case-control samples (295 African American matched pairs and 366 white matched pairs) (119). BTNL2 moderately influences disease risk (ORs of 1.6 in heterozygotes and 2.8 in homozygotes) and seems to be somewhat less associated with sarcoidosis in African Americans compared with whites.

One question regarding BTNL2 as a sarcoidosis risk factor is whether it is independent of HLA-DRB1 risk alleles. HLADRB1 and BTNL2 are in linkage disequilibrium. HLA DRB lies about 180 kb centromeric to BTNL2. Based on regression models, BTNL2 seems to be an independent risk factor (116, 119). In the case of African Americans, where the BTNL2 conferred sarcoidosis risk is less significant than in whites, a negative interaction with HLA-DR seems to exist (119).

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 less than 0.05 with the strongest linkage signal on chromosome 5 (120). Fine mapping studies indicated a sarcoidosis susceptibility gene on chromosome 5q11.2 and a gene protective effect for sarcoidosis on 5p15.2 (121). Although choosing to study African Americans to uncover sarcoidosis susceptibility genes makes sense because African Americans are more commonly and severely affected and have affected family members more often than whites, a disadvantage is that African Americans are admixed with white and other populations to varying degrees with possible admixture among our participating centers ranging from 12% in South Carolina to 20% in New York (122).

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 (123). 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. Additional studies are underway to evaluate candidate genes from these linked regions.

Markers on chromosome 16, close to the Blau syndrome/IBD locus on chromosome 5 near the cytokine gene cluster and on chromosome 2 near the NRAMP locus, did not suggest linkage to sarcoidosis in either genome scan.

A genomewide association study in which high throughput genotyping methods are used to genotype a dense set of SNPs across the genome has yet to be performed in sarcoidosis. A significant advantage of this approach is that association studies are more powerful than affected sib pair methods of linkage analysis (124). Generally, large samples sizes (i.e., several hundred to a few thousand) will likely be required if several genes with modest affect are involved. Groups in Europe and in the United States are preparing to carry out this approach.

Outlook

Sarcoidosis likely develops from complex interactions between environmental agents and alleles of many genes. The environmental causes remain unknown, and over 80 yr has elapsed since Martenstein first reported that sarcoidosis can occur in siblings (125). Since then, HLA and other candidate genes have been 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.

Emerging technologies and advances in genomics and proteomics are bringing us closer to answering three common questions patients ask: What causes sarcoidosis? Why did I get it? How will it affect my health? Methods such as multiplexed liquid chromatography and nano–high-performance liquid chromatography coupled to electrospray or matrix-assisted laser desorption/ionization mass spectrometry are expected to lead us to the inciting sarcoid antigens. Gene expression profiling in BALF and blood carried out at the time of presentation will likely allow us to better predict disease resolution or progression and response to treatment. Functional analysis of the candidate genes identified by linkage analysis and whole genome association studies will provide insight into pathogenesis and disease risk.

Conflict of Interest Statement: Neither author has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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