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Sarcoidosis and a MIF gene polymorphism: a case-control study in an Irish Population.

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ABSTRACT

Macrophage Migration Inhibitory Factor is a key pro-inflammatory mediator. A 5-CATT repeat functional polymorphism within the promoter of the gene associated with lowest promoter activity. Our hypothesis is that patients exhibiting a 5-CATT allele would have a less aggressive inflammatory response with an associated less severe clinical phenotype in sarcoidosis.

Irish Caucasian Sarcoidosis patients (n=173) followed up for 1 to 39 years and a control group (n=166) were genotyped for the CATT repeat polymorphism. Disease severity at diagnosis and currently was assessed by the presence of thoracic and extrathoracic symptoms, Erythema Nodosum, radiographic interstitial changes (Chest X-ray score equal to stage II or greater or high resolution computerised tomography confirmed), pulmonary function tests, steroid use, and current erythrocyte sedimentation rate, c-reactive protein and angiotensin converting enzyme levels.

In an Irish population no evidence was found of a significant association between either sarcoidosis susceptibility and disease

severity and the 5-CATT repeat functional polymorphism in the Macrophage migration inhibitory gene.

This work found no significant association between the MIF 5-CATT repeat Macrophage Migration Inhibitory Factor gene polymorphism and sarcoidosis and would not support the overriding role for Macrophage Migration Inhibitory Factor in driving sarcoidosis pathogenesis.

Keywords:

Macrophage Migration Inhibitory Factor, MIF, Polymorphism, Sarcoidosis.

INTRODUCTION

Sarcoidosis is a multisystemic granulomatous disorder of unknown aetiology, which can affect any organ system, however, lung involvement is seen in more than 90% of patients. Common extrathoracic sites include lymph nodes, skin, and eyes (1, 2). The general concept has emerged that Sarcoidosis results from the exposure of genetically susceptible individuals to specific environmental agents as yetunknown (1, 2). Genetic susceptibility to sarcoidosis is supported by familial clustering (3) and a higher prevalence of disease in first-degree relatives (4). In Ireland the prevalence of Sarcoidosis cases among siblings is 2.4% (5).

Macrophage migration inhibitory factor (MIF) is a key pro-inflammatory mediator (6). It is secreted by a variety of pulmonary cells including monocyte/macrophages (7), endothelial cells and various epithelial cells including bronchial epithelium (8). Culture supernatants of cutaneous granulomas from patients with sarcoidosis contain MIF (9). It contributes towards an excessive inflammatory response both directly via an induction of pro-inflammatory cytokine secretion, including TNF-a and IL-8 (10) and indirectly through its

ability to over-ride the anti-inflammatory activity of glucocorticoids (8). MIF is an important regulator of adaptive immunity. T cells constitutively express MIF (11). Expression studies using Th1 and Th2 subsets found that while both subsets of T cells express MIF, secretion is predominantly in activated Th2 clones (11). These data would favour the role for MIF in the development of Th2-driven antibody production.

MIF is implicated in a wide number of acute and chronic immune and inflammatory diseases including ARDS (Acute Respiratory Distress Syndrome) (8), Asthma (12), septic shock (10), and rheumatoid arthritis (13). Anti-MIF neutralising antibodies reduce the production of TNF alpha and IL-8 (8, 10) highlighting the potential role of an anti-MIF strategy as a therapeutic target clinically, in inflammatory lung disease. The potential role of MIF in granulomatous disease was highlighted by the ability of an anti-MIF monoclonal antibody strategy to attenuate granulomatous inflammatory bowel disease in an animal model of Crohn's disease (13).

We recently described a functional CATT repeat promoter polymorphism in the MIF gene (14). Individuals were identified that were homozygous or heterozygous for 5, 6, 7 or 8 CATT repeats at position -794, and these allelic variations in MIF were designated 5-

CATT, 6-CATT, 7-CATT and 8-CATT. The 5-CATT repeat allele exhibited lowest MIF promoter activity in vitro. In a cohort of rheumatoid arthritis patients, patients homo- or heterozygous for the 5-CATT allele had less aggressive disease ($P<0.02$) (14).

The passage from granuloma to fibrosis is not well understood in Sarcoidosis. TH1 cytokines (IL-2, IL-12, and IFN-g) are likely to favour granulomatous formation (15). A shift from a Th1 to a Th2 phenotype (IL-4, IL-5, and IL-10) may be important for persistent and progressive fibrotic disease (16).

Given MIF's pro-inflammatory role in inflammation, its ability both to over-ride the anti-inflammatory action of glucocorticoids and to drive a Th2 phenotype, we believe that the MIF gene may potentially modify the clinical course in Sarcoidosis.

Our hypothesis is that sarcoidosis patients exhibiting the 5-CATT repeat polymorphism in the MIF gene, and consequently less MIF promoter activity, would have a less aggressive inflammatory response and consequently milder clinical disease.

METHODS

Study Population

In a case-control study, consecutive sarcoidosis patients (n=176) from a pulmonary specialist clinic at St. Vincent's University Hospital, Dublin provided genomic DNA for analysis. All patients had been followed up for 1-39 years. A cohort of Irish Caucasian healthy volunteers (n=166) was recruited as a control group. The control group has no active medical problems, no known history of lung disease including asthma and was on no regular medications including inhaled medication. Informed written consent was obtained from all participants. The ethics committee of St. Vincent's University Hospital approved the study.

All sarcoidosis patients were Caucasian in origin, aged over 18 years. Diagnosis was confirmed by either tissue biopsy (90%) or a compatible clinical presentation with radiographic evidence of bilateral hilar adenopathy (BHL), and bronchoalveolar lavage (BAL) findings consistent with a diagnosis of sarcoidosis (i.e. CD4/CD8 ratio of 3.5 or greater) (17). In the non-tissue proven group (10%) all were followed up for at least 1 year with no other medical condition that could

explain the clinical course. All were clinically stable at the time of recruitment, defined as no recent deterioration in symptoms, pulmonary function tests, or a change in any prescribed medication including oral and inhaled corticosteroids, within the previous six weeks.

Assessment of disease severity.

At recruitment a pulmonary physician performed a detailed direct patient interview and results were crosschecked with medical record review. Subjectively disease severity was determined by the presence of thoracic (including cough, wheeze, shortness of breath) and extrathoracic (including anergy, eye, lymph node, joint, skin, cardiac, or neurological anomalies) symptoms at diagnosis and at review (currently). Objectively intrathoracic changes were evaluated by radiographic and pulmonary function tests, all performed at this centre, at diagnosis and on study enrollment. Chest X-rays (staged 0 to IV i.e. normal to fibrosis) (18) and/or High Resolution Computerised Tomography (HRCT) determined the presence or absence of a pulmonary infiltrate or fibrosis. Radiologically bilateral hilar adenopathy (Stage I) alone has the best prognosis (19), and was specifically recorded. Forced expiratory volume in 1 second (FEV1),

forced vital capacity (FVC), and Transfer Factor (DLco) were measured at recruitment and expressed as a percentage (%) predicted, as per international guidelines (20, 21). Initial values were obtained from the medical records. Current biochemical markers of disease activity were determined by erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) (22), Lymphocyte count, serum calcium, and angiotensin converting enzyme (ACE). Abnormal liver function tests (LFTs) defined as a persistent elevation above the upper limit of normal serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT) or bilirubin and highest ACE level were also recorded.Oral corticosteroid treatment ever and/or currently was noted, as was duration of usage, and current dose. A family history of sarcoidosis was defined as sarcoidosis in a first or second degree relative was also established.

Identification of the Polymorphism

Genomic DNA was extracted from anticoagulated whole blood collected in EDTA, from cases and controls using QIAamp DNA Blood Mini Kits (QIAGEN Ltd. U.K.). PCR amplification of the polymorphic region for the CATT repeat polymorphism was performed using the forward

primer MIF-Forward (5'-TGC AGG AAC CAA TAC CCA TAG G-3') and a TET fluorescent reverse primer MIF-Reverse (TET lab5' –AAT GGT AAA CTC GGG GAC-3) (Microsynth) (16). TET labeled amplicons were resolved using an ABI 310 Genetic Analyser, as described previously (14). DNA from previously genotyped homozygous individuals, verified by direct sequencing, was used to generate control amplicons for size calibration.

Statistical analysis

For the purpose of statistical analysis subjects were designated MIF5+ (homo- or heterozygous for the MIF 5-repeat allele) or MIF5-. Continuous variables were analysed using Student's t test for normally distributed, or Mann Whitney testing for non-parametric data. Data analysis wasperformed using SPSS statistical software (version 11). A p value <0.05 was taken as the level for significance.

RESULTS

Clinical characteristics relating to the sarcoidosis patients are outlined in Table 1. Sex did not differ significantly between cases and controls with 46% and 50% male respectively (Chi-squared test: p=0.428). The median age of cases at diagnosis and controls at recruitment was 31 and 26 years respectively. Cases had been followed up for a median of 12 years (range 1-39 years).

Table1. Clinical characteristics of Sarcoidosis cohort.

		Yes	No
Erythema Nodosum			
At diagnosis	%	25.3	74.7
Thoracic Symptoms			
At diagnosis	%	54.0	46.0
At review	%	10.4	89.6
Extrathoracic Symptoms			
At diagnosis	%	77.9	22.1
At review	%	17.3	82.7
Oral Steroids			
Ever	%	64.0	36.0
At review	%	29.5	70.5
Radiographic Infiltrate			
At diagnosis	%	49.6	50.4
At review	%	40.4	58.6
BHL alone			
At diagnosis	%	43.6	51.6
At review	%	22.8	77.2
Family History of Sarcoid			
	%	12.3	87.7
Smoking History			
Ever	%	26.0	74.0
At review	%	5.4	94.6

There was no significant difference in MIF frequencies with 42.7% (n=71/166) of controls and 45.5% (n=80/176) of sarcoidosis patients possessing the 5-CATT repeat allele (Chi-squared test, p=0.85) (Table 2).

Table2. MIF CATT genotype frequencies in cases and controls.

	MIF Genotype								
	5,5	5,6	5,7	5,8	6,6	6,7	6,8	7,7	7,8
Control (n)	7	51	13	0	70	20	1	3	1
(%)	4.2	30.7	7.8	0	42.2	12	0.6	1.8	0.6
Sarcoid (n)	9	58	12	1	69	23	1	3	0
(%)	5.1	33	6.8	0.6	39.2	13.1	0.6	1.7	0

No significant difference in genotype frequencies between sarcoidosis cases and controls (Chi Squared test: p=0.85)

In the sarcoidosis cohort there was no significant difference in the MIF 5^+ carrier status between males (44.4%) and females (46.3%) (Chi-squared test, p=0.8). The age (years) at diagnosis did not differ between MIF 5^+ carriers (32.82; standard deviation (SD) 9.99) and MIF 5^- (34.05; SD 10.99) (t test, p=0.447) nor did the current mean age (44.95;SD 12.42 vs. 47.42; SD 12.52) (t test, p=0.193).

Table 3 summarises the lack of any significant association between clinical symptoms (including the presence of Erythema Nodosum), radiographic changes, family history, oral corticosteroid use and $MIF5^{+}$ carrier status, either at diagnosis or currently. There is no difference in duration (Mann Whitney, P=0.21) or current dose of oral corticosteroids (Mann Whitney, P=0.73). There is no significant association between $MIF5^{+}$ carrier status and pulmonary function (Table 4) or ESR (p=0.082), CRP (p=0.492), Calcium (p=0.429), ACE (p=0.932), Highest ACE (p=0.414), Lymphocyte count (p=0.585) and Percentage with abnormal liver function (p=0.847).

Table 3. MIF CATT genotypes and disease severity.

		MIF5$^+$	MIF5$^-$	Chi-squared test p-value
Erythema Nodosum				
At diagnosis	%	24.3	26.3	0.783
Thoracic Symptoms				
At diagnosis	%	50.0	57.7	0.358
Currently	%	12.5	8.6	0.402
Extrathoracic symptoms				
At diagnosis	%	82.9	73.4	0.166
Currently	%	21.7	14.0	0.208
Oral Steroids				
Ever	%	66.3	62.1	0.569
Currently	%	30.0	29.2	0.904
Radiographic Infiltrate				
At diagnosis	%	46.8	52.1	0.374
Currently	%	40.8	40.0	0.918
BHL alone				
At diagnosis	%	48.4	39.4	0.299
At review	%	40.8	40.0	0.918
Family History Sarcoid				
	%	15.8	9.3	0.210
Smoking History				
Ever	%	22.5	29.1	0.359
At review	%	8.5	2.6	0.120

No significant association between MIF5$^+$ carrier status and disease severity.

Table 4. MIF CATT genotypes and Pulmonary Function.

		MIF5^{+}	MIF5^{-}	Mann Whitney test p-value
FEV1 % predicted				
At diagnosis	n	54	55	0.732
	Median	99.5	101	
	Centiles*	65-127	62-118	
At review	n	74	80	0.921
Currently	Median	96	96	
	Centiles*	53-127	54-127	
FVC % predicted				
At diagnosis	n	56	54	0.240
	Median	97.5	100	
	Centiles*	59-129	74-122	
Currently	n	74	80	0.738
	Median	99.5	100	
	Centiles*	62-128	74-122	
DLco % predicted				
At diagnosis	n	59	57	0.574
	Median	82.1	83,0	
	Centiles*	53-117	53-110	
Currently	n	72	79	0.220
	Median	81.2	87.0	
	Centiles*	45-113	55-106	

No significant association between MIF5+ carrier status and pulmonary function.

Centiles* (5^{th} to 95^{th} centiles).

DISCUSSION

In this case-control study of an Irish population with sarcoidosis we found no significant association between the presence of the 5-CATT MIF-promoter polymorphism (MIF 5+) and milder clinical disease. In particular no association was demonstrated between those patients heterozygous for the 5-CATT polymorphism and disease activity, in contrast to other studies of patients with chronic pulmonary and non-pulmonary inflammatory diseases (eg Asthma, Cystic Fibrosis and Rheumatoid Arthritis (RA)). (14,23,24). With reference to the role of patients homozygous for the 5-CATT polymorphism and disease activity, further work in a larger patient cohort will be required to definitively address this.

Recent data has described a G to C single nucleotide polymorphism (SNP) at position –173 of the MIF gene, which has been found in vitro to be associated with increased levels of MIF gene transcription (25) and an association between the development of Sarcoidosis in patients with Erythema Nodosum (EN) and this SNP has been reported in a Spanish cohort (26). Contrary to our expectation possession of a 5-CATT repeat allele was not associated with a decreased risk in Irish sarcoidosis patients presenting with EN. Given that genetic and environmental background can also affect genetic associations (2),

ethnic specific associations of given genes and polymorphisms with susceptibility to sarcoidosis are possible. This is highlighted both by conflicting reports in other candidate genes and genetic polymorphism studies in various ethnicities (27, 28), and by comparing the percentage of EN in our Caucasian cohort (25.3%) with a recent American Caucasian group (7.6%) (29).

This is the first paper to examine the role of MIF in pulmonary sarcoidosis, particularly with regard to severity and progression of disease. The precise mechanisms, which regulate a shift from a granulomatous to a fibroproliferative disease process, remains unknown, however, it may represent shifting from a Th1 to a Th2 phenotype (16). Biologically MIF has a plausible role in this process. Cutaneous granulomas from patients with sarcoidosis contain MIF (9). It contributes towards an excessive inflammatory response (8, 10). T cells constitutively express MIF and enhanced secretion has been shown in activated Th2 clones (11). With this in mind we specifically radiologically classified our sarcoidosis into two groups: those with milder radiological disease compared to those with interstitial or fibrotic changes. Contrary to our expectation possession of a 5-CATT repeat allele was not associated with less interstial/fibrotic radiographic disease. Patients presenting with bilateral hilar

adenopathy alone have a good prognosis (19). There was no association between MIF5+ carrier status and BHL alone on Chest X-ray. In addition no association was found with selected pulmonary function parameters, symptomatology or corticosteroid usage.

The identification of key regulatory cytokines that attenuate aggressive disease in sarcoidosis is of paramount importance in the design of specific targeted therapies (30). We have previously highlighted that specific targeted anti-MIF antibody strategies offer a potential novel therapeutic approach to inflammatory disease based on individual patient MIF genotype, in Rheumatoid arthritis (14). We have found no significant association between our MIF gene polymorphisms and susceptibility, severity or in sarcoidosis. Our group is representative of chronic sarcoidosis with sixty four percent of the sarcoidosis cohort in total were at some point on steroids and 49.6% having confirmed lung infiltrates radiologically at diagnosis. The data was assessed at two time points (diagnosis and review) and our enrolled patients have been followed up for a median of 12 years. While our data would not support the concept of MIF having an overriding effect in driving aggressive disease in sarcoidosis. Our study of sarcoidosis patients had significant numbers of individuals who were heterozygote for the 5-CATT polymorphism, but a relatively smaller number of homozygous

individuals (n=9 (5.1%) sarcoidosis patients and n=7 (4.2%) controls). Due to the small number of $MIF5^{+}$ homozygotes, it was not possible to obtain reliable estimates of gene dose effects. A further larger cohort study would be required to definitively address this.

It is interesting to note that the finding of 12.3% of patients reporting a first or second degree relative with sarcoidosis correlates with a previous study from the department 20 years ago where 9.6% reported at least one sibling with sarcoidosis (5).

In any genetic association study there is always the concern that there may be confounding because cases and controls are not well matched. This was a well-matched group for age and sex. Our control group is from the same population ethnically and given that the genotype frequencies did not differ between cases and controls, population stratification is unlikely (31).

In conclusion this study did not show an association between the 5-CATT functional repeat polymorphism in the MIF gene and sarcoidosis in an Irish Caucasian population.

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REFERENCES

1. Costabel U. Sarcoidosis: clinical update. Eur Respir J Suppl. 2001 Sep;32:56s-68s.

2. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med. 1999 Aug;160(2):736-55.

3. Rybicki BA, Iannuzzi MC, Frederick MM, Thompson BW, Rossman MD, Bresnitz EA, Terrin ML, Moller DR, Barnard J, Baughman RP, DePalo L, Hunninghake G, Johns C, Judson MA, Knatterud GL, McLennan G, Newman LS, Rabin DL, Rose C, Teirstein AS, Weinberger SE, Yeager H, Cherniack R; ACCESS Research Group. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). Am J Respir Crit Care Med. 2001 Dec 1;164(11):2085-91.

4. Rybicki BA, Kirkey KL, Major M, Maliarik MJ, Popovich J Jr, Chase GA, Iannuzzi MC. Familial risk ratio of sarcoidosis in African-American sibs and parents. Am J Epidemiol. 2001 Jan 15;153(2):188-93.

5. Brennan NJ, Crean P, Long JP, Fitzgerald MX. High prevalence of familial sarcoidosis in an Irish population. Thorax. 1984 Jan;39(1):14-8.

6. Donnelly SC, Bucala R. Macrophage migration inhibitory factor: a regulator of glucocorticoid activity with a critical role in inflammatory disease. Mol Med Today 1997;3:502-507.

7. Bernhagen J, Bacher M, Calandra T, Metz CN, Doty SB, Donnelly T, Bucala R. An essential rolefor macrophage migration inhibitory factor in tuberculin delayed –type hypersensitivity reaction. J Exp Med 1996;183:277-282.

8. Donnelly SC, Haslett C, Reid PT, Grant IS, Wallace WA, Metz CN, Bruce LJ, Bucala R. Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat Med 1997;3;320-323.

9. Campbell PB, Kataria YP, Tolson TA. In vitro production of inhibitors of monocyte locomotion by the granuloma of sarcoidosis. Am Rev Respir Dis. 1984 Sep;130(3):417-23.

10. Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L, Heumann D, Mannel D, Bucala R, Glauser MP. Protection from septic

shock by neutralization of macrophage migration inhibitory factor. Nat Med 2000 ;6[2]: 164-170.

11. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, Gemsa D, Donnelly T, Bucala R. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc Natl Acad Sci U S A. 1996 Jul 23;93(15):7849-54.

12. Rossi AG, Haslett C, Hirani N, Greening AP, Rahman I, Metz CN, Bucala R, Donnelly SC. Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. J Clin Invest. 1998 Jun 15;101(12):2869-74.

13. de Jong YP, Abadia-Molina AC, Satoskar AR, Clarke K, Rietdijk ST, Faubion WA, Mizoguchi E, Metz CN, Allsahli M, ten Hove T, Keates AC, Lubetsky JB, Farrell RJ, Michetti P, van Deventer SJ, Lolis E, David JR, Bhan AK, Terhorst C. Development of chronic colitis is dependent on the cytokine MIF. Nat Immunol. 2001 Nov;2(11):1061-6.

14. Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ, Wolfe F, Gregersen PK, Bucala R. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated

with disease severity in rheumatoid arthritis. Genes Immun. 2002 May;3(3):170-6.

15. Moller DR. Cells and cytokines involved in the pathogenesis of sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 1999 Mar;16(1):24-31.

16. Kunkel SL, Lukacs NW, Strieter RM, Chensue SW. Th1 and Th2 responses regulate experimental lung granuloma development. Sarcoidosis Vasc Diffuse Lung Dis. 1996 Sep;13(2):120-8.

17. Costabel U. CD4/CD8 ratios in bronchoalveolar lavage fluid: of value for diagnosing sarcoidosis? Eur Respir J. 1997 Dec;10(12):2699-700.

18. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. Br Med J. 1961 Nov 4;5261:1165-72.

19. Hillerdal G, Nou E, Osterman K, Schmekel B. Sarcoidosis: epidemiology and prognosis. A 15-year European study. Am Rev Respir Dis. 1984 Jul;130(1):29-32.

20. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. Am Rev Respir Dis. 1981 Jun;123(6):659-64.

21. American Thoracic Society. Standardization of Spirometry, 1994 Update. Am J Respir Crit Care Med. 1995 Sep;152(3):1107-36.

22. Drent M, Wirnsberger RM, de Vries J, van Dieijen-Visser MP, Wouters EF, Schols AM. Association of fatigue with an acute phase response in sarcoidosis. Eur Respir J. 1999 Apr;13(4):718-22.

23. Mizue Y, Ghani S, Leng L, McDonald C, Kong P, Baugh J, Lane SJ, Craft J, Nishihira J, Donnelly SC, Zhu Z, Bucala R. Role for macrophage migration inhibitory factor in asthma. Proc Natl Acad Sci USA. 2005;102:14410-5.

24. Plant BJ, Gallagher CG, Bucala R, Baugh JA, Chappell S, Morgan L, O'Connor CM, Morgan K, Donnelly SC. Cystic fibrosis, disease severity, and a macrophage migration inhibitory factor polymorphism. Am J Respir Crit Care Med. 2005;172:1412-5.

25. Donn RP, Shelley E, Ollier WE, Thomson W; British Paediatric Rheumatology Study Group. A novel 5'-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2001 Aug;44(8):1782-5.

26. Amoli MM, Donn RP, Thomson W, Hajeer AH, Garcia-Porrua C, Lueiro M, Ollier WE, Gonzalez-Gay MA. Macrophage migration inhibitory factor gene polymorphism is associated with sarcoidosis in biopsy proven erythema nodosum. J Rheumatol. 2002 Aug;29(8):1671-3.

27. Rybicki BA, Maliarik MJ, Poisson LM, Iannuzzi MC. Sarcoidosis and granuloma genes: a family-based study in African-Americans. Eur Respir J. 2004 Aug;24(2):251-7.

28. Pietinalho A, Furuya K, Yamaguchi E, Kawakami Y, Selroos O. The angiotensin-converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients. Eur Respir J. 1999 Apr;13(4):723-6.

29. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R; Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001 Nov 15;164(10 Pt 1):1885-9.

30. Baughman RP, Lower EE. Infliximab for refractory sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2001 Mar;18(1):70-4.

31. Cardon LR,Palmer LJ. Population stratification and spurious allelic association. Lancet. 2003 Feb 15;361(9357):598-604.