Sarcoidosis and MIF gene polymorphism: a case–control study in an Irish population

B.J. Plant*, S. Ghani*, M.J. O'Mahony*, L. Morgan[#], C.M. O'Connor*, K. Morgan[#], J.A. Baugh* and S.C. Donnelly*

ABSTRACT: Macrophage migration inhibitory factor is a key pro-inflammatory mediator. A 5-CATT repeat functional polymorphism within the promoter of the gene was previously associated with the lowest promoter activity. It was hypothesised 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 1–39 yrs and a control group (n=166) were genotyped for the CATT repeat polymorphism. Disease severity at the time of diagnosis and at the time of elaboration of the present study was assessed by the presence of thoracic and extrathoracic symptoms, erythema nodosum, radiographic interstitial changes (chest radiograph score equal to stage II or greater, or high-resolution computed tomography confirmed), pulmonary function tests, steroid use, erythrocyte sedimentation rate, C-reactive protein and angiotensin-converting enzyme levels.

In the Irish population studied, 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.

The present study found no significant association between the 5-CATT repeat macrophage migration inhibitory factor gene polymorphism and sarcoidosis, and did not support the overriding role for macrophage migration inhibitory factor in driving sarcoidosis pathogenesis.

KEYWORDS: Macrophage migration inhibitory factor, polymorphism, sarcoidosis

Sarcoidosis is a multisystemic granulomatous disorder of unknown aetiology, which can affect any organ system. However, lung involvement is seen in >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 that are as yet unknown [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, such as 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 tumour necrosis factor (TNF)- α and interleukin (IL)-8 [10], and indirectly, through its ability to override the anti-inflammatory activity of glucocorticoids [8]. MIF is an important regulator of adaptive immunity. T-cells constitutively express MIF [11]. Expression studies using T-helper cell (Th) types 1 and 2 subsets found that, while both subsets of T-cells express MIF, secretion occurs 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 acute respiratory distress syndrome [8], asthma [12], septic shock [10] and rheumatoid arthritis (RA) [13]. Anti-MIF neutralising antibodies reduce the production of TNF- α and IL-8 [8, 10], highlighting the potential role of an anti-MIF strategy as a clinical therapeutic target 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

AFFILIATIONS

*The Conway Institute of Biomolecular and Biomedical Research, School of Medicine & Medical Science, University College Dublin, Dublin, Ireland.

[#]Institute of Genetics, QMC, University of Nottingham, Nottingham, UK.

CORRESPONDENCE

S.C. Donnelly
Medicine and Therapeutics
The Education Research Centre
St. Vincent's University Hospital
Elm Park
Dublin 4
Ireland
Fax: 35 312773750
E-mail: seamas.donnelly@ucd.ie

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STATEMENT OF INTEREST

None declared.

inflammatory bowel disease in an animal model of Crohn's disease [13].

The present authors 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. These allelic variations in MIF were designated 5-CATT, 6-CATT, 7-CATT and 8-CATT, respectively. The 5-CATT repeat allele exhibited the lowest MIF promoter activity *in vitro*. In a cohort of RA patients, individuals homo- or heterozygous for the 5-CATT allele presented a 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 interferon- γ) 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 override the anti-inflammatory action of glucocorticoids and to drive a Th2 phenotype, the present authors believe that the MIF gene may potentially modify the clinical course in sarcoidosis.

It was hypothesised 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, therefore, present a 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, Ireland) provided genomic DNA for analysis. All patients had been followed up for 1–39 yrs. A cohort of Irish Caucasian healthy volunteers ($n=166$) was recruited as a control group. The control group had no active medical problems, no known history of lung disease (including asthma) and was on no regular medication (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 and of >18 yrs of age. Diagnosis was confirmed by either tissue biopsy (90%) or a compatible clinical presentation with radiographic evidence of bilateral hilar adenopathy (BHL) and bronchoalveolar lavage findings consistent with a diagnosis of sarcoidosis (*i.e.* CD4/CD8 ratio ≥ 3.5) [17]. In the non-tissue proven group (10%), all patients were followed up for ≥ 1 yr 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 prescribed medication, including oral and inhaled corticosteroids, within the previous 6 weeks.

Assessment of disease severity

At recruitment, a pulmonary physician performed a detailed direct patient interview and the results were cross-checked

with a medical record review. Subjectively, disease severity was determined by the presence of thoracic (including cough, wheeze or shortness of breath) and extrathoracic (including anergy and eye, lymph node, joint, skin, cardiac or neurological anomalies) symptoms at diagnosis and at review. Objectively, intrathoracic changes were evaluated by radiographic and pulmonary function tests, performed at St. Vincent's University Hospital at diagnosis and on study enrolment. Chest radiographs (staged 0–IV, *i.e.* normal to fibrosis) [18] and/or high-resolution computed tomography determined the presence or absence of a pulmonary infiltrate or fibrosis. Radiological BHL (stage I) alone has the best prognosis [19] and was specifically recorded. Forced expiratory volume in one second, forced vital capacity and transfer factor were measured at recruitment and expressed as % predicted, according to international guidelines [20, 21]. Initial values were obtained from the medical records. 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, (defined as a persistent elevation above the upper limit of normal serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ -glutamyl transferase or bilirubin) and highest ACE level were also recorded. Oral corticosteroid treatment ever and/or at the time of the study was noted, as was duration of usage and dose. A family history of sarcoidosis, 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, Crawley, UK). 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 tetrachlorofluorescein (TET) fluorescent reverse primer MIF-Reverse (TET lab5'-AAT GGT AAA CTC GGG GAC-3; Microsynth, Balgach, Switzerland) [16]. TET-labelled amplicons were resolved using an ABI 310 Genetic Analyser (Perkin Elmer-Applied Biosystems, CA, USA), as described elsewhere [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-CATT repeat allele) or MIF5-. Categorical variables, including steroid usage, radiographic change, family history of sarcoidosis and smoking history were compared in the MIF5+ and MIF5- groups using Chi-squared testing. Continuous variables were analysed using unpaired t-tests for normally distributed or Mann-Whitney testing for nonparametric data. 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% males, respectively

TABLE 1 Clinical characteristics of the sarcoidosis cohort

	Yes	No
EN		
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 sarcoidosis	12.3	87.7
Smoking history		
Ever	26.0	74.0
At review	5.4	94.6

Data are presented as %. EN: erythema nodosum; BHL: bilateral hilar adenopathy.

($p=0.428$). The median age of cases at diagnosis and controls at recruitment was 31 and 26 yrs, respectively. Cases had been followed up for a median of 12 yrs (range 1–39 yrs).

There was no significant difference in MIF frequencies, with 42.7% of controls and 45.5% of sarcoidosis patients possessing the 5-CATT repeat allele ($p=0.85$; table 2).

In the sarcoidosis cohort there was no significant difference in the MIF5+ carrier status between males (44.4%) and females (46.3%; $p=0.8$). Age (yrs) at the time of diagnosis did not differ between MIF5+ carriers (mean $\pm$ SD = 32.82 ± 9.99) and MIF5- (34.05 ± 10.99 ; $p=0.447$), nor did the mean age at the time of elaboration of the present study (44.95 ± 12.42 versus 47.42 ± 12.52 ; $p=0.193$).

Table 3 summarises the lack of significant association between clinical symptoms (including the presence of erythema nodosum (EN)), radiographic changes, family history, oral corticosteroid use and MIF5+ carrier status, either at the time of

TABLE 3 Macrophage migration inhibitory factor (MIF) CATT genotypes and disease severity

	MIF5+	MIF5-	p-value [#]
EN			
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 of sarcoidosis	15.8	9.3	0.210
Smoking history			
Ever	22.5	29.1	0.359
At review	8.5	2.6	0.120

Data are presented as %, unless otherwise stated. No significant association between MIF5+ carrier status and disease severity was observed. EN: erythema nodosum; BHL: bilateral hilar adenopathy. #: obtained using Chi-squared tests.

diagnosis or at the time of elaboration of the present study. There is no difference in duration of treatment ($p=0.21$) or dose of oral corticosteroids ($p=0.73$). There is no significant association between MIF5+ carrier status and pulmonary function (table 4) or ESR ($p=0.082$), CRP ($p=0.492$), serum calcium ($p=0.429$), ACE ($p=0.932$), highest ACE ($p=0.414$), lymphocyte count ($p=0.585$) and percentage with abnormal liver function ($p=0.847$).

DISCUSSION

In the present case-control study of an Irish population with sarcoidosis, no significant association was found between the presence of the 5-CATT MIF-promoter polymorphism (MIF5+) and milder clinical disease. In particular, no association was demonstrated between those patients heterozygous for the

TABLE 2 Macrophage migration inhibitory factor (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	7 (4.2)	51 (30.7)	13 (7.8)	0 (0)	70 (42.2)	20 (12)	1 (0.6)	3 (1.8)	1 (0.6)
Sarcoidosis	9 (5.1)	58 (33)	12 (6.8)	1 (0.6)	69 (39.2)	23 (13.3)	1 (0.6)	3 (1.7)	0 (0)

Data are presented as n (%). No significant difference in genotype frequencies between sarcoidosis cases and controls was observed ($p=0.85$).

TABLE 4 Macrophage migration inhibitory factor (MIF) CATT genotypes and pulmonary function

		MIF5+	MIF5-	p-value [#]
FEV1 % pred				
At diagnosis	n	54	55	0.732
	Median	99.5	101	
	Centiles [†]	65–127	62–118	
At review	n	74	80	0.921
	Median	96	96	
	Centiles [†]	53–127	54–127	
FVC % pred				
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 % pred				
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 was observed. FEV₁: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; DL_{CO}: transfer factor. [#]: obtained using Mann-Whitney tests; [†]: 5th to 95th centiles.

5-CATT polymorphism and disease activity, in contrast to other studies of patients with chronic pulmonary and non-pulmonary inflammatory diseases (e.g. asthma, cystic fibrosis and 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]. An association between the development of sarcoidosis in patients with EN and this SNP has been reported in a Spanish cohort [26]. Contrary to the present authors' 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], ethnicity-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 the present Caucasian cohort (25.3%) with a recent American Caucasian group (7.6%) [29].

The present study is the first to examine the role of MIF in pulmonary sarcoidosis, particularly with regard to severity and progression of disease. The precise mechanism, which

regulates 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, the sarcoidosis patients of the present study were specifically radiologically classified into two groups: those with milder radiological disease and those with interstitial or fibrotic changes. Contrary to the present authors' expectations, possession of a 5-CATT repeat allele was not associated with less interstitial/fibrotic radiographic disease. Patients presenting with BHL alone have a good prognosis [19]. There was no association between MIF5+ carrier status and BHL alone on chest radiographs. In addition, no association was found with selected pulmonary function parameters, symptomatology or corticosteroid use.

The identification of key regulatory cytokines that attenuate aggressive disease in sarcoidosis is of paramount importance in the design of specific targeted therapies [30]. The present authors 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 RA [14]. No significant association has been found between MIF gene polymorphisms and susceptibility and severity of the disease in sarcoidosis. The present group of patients is representative of chronic sarcoidosis, with 64% of the cohort having been on steroids at some point and 49.6% having confirmed lung infiltrates radiologically at diagnosis. The data was assessed at two time-points (diagnosis and review) and the enrolled patients have been followed up for a median of 12 yrs. The present data would not support the concept of MIF having an overriding effect in driving aggressive disease in sarcoidosis. The present study of sarcoidosis patients had significant numbers of individuals who were heterozygous for the 5-CATT polymorphism, but a relatively smaller number of individuals who were homozygous (nine (5.1%) sarcoidosis patients and seven (4.2%) controls). Due to the small number of MIF5+ homozygotes, it was not possible to obtain reliable estimates of gene dose effects. A 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 by BRENNAN *et al.* [5], in which 9.6% reported at least one sibling with sarcoidosis.

In any genetic association study there is always the concern that there may be a degree of inaccuracy due to cases and controls not being well matched. The present cohort was a well-matched group for age and sex. The present 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, the present study did not show an association between the 5-CATT functional repeat polymorphism in the macrophage migration inhibitory factor gene and sarcoidosis in an Irish Caucasian population.

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