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Fas promoter polymorphisms: genetic predisposition to sarcoidosis in African-Americans

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Abstract

Apoptosis may perpetuate some forms of inflammation. Of the apoptotic pathway proteins, Fas is particularly overexpressed in sarcoidosis. We hypothesized that Fas promoter single nucleotide polymorphisms (SNPs) contribute to the development and severity of sarcoidosis. Associations of known Fas promoter SNPs (−670, −690 and −1377) and deduced haplotypes with sarcoidosis and sarcoidosis severity were evaluated using matched case–control ($n = 656$ pairs) and case–comparison ($n = 656$) studies, respectively, using conditional logistic regression. Hardy–Weinberg equilibrium was confirmed for all three polymorphisms in African-Americans (AA), and for the −670 and −1377 in whites. Genotype and allele frequencies were significantly different between whites and AA. Race-stratified analysis revealed that a common haplotype, −1377G/−690T/−670G, was associated with sarcoidosis [odds ratio (OR) = 1.78, $P = 0.05$] only in AA. The haplotype −1377G/−690C/−670A was negatively associated with sarcoidosis (OR = 0.39, $P = 0.03$) only in AA. In conclusion, the consistency of these findings suggests that Fas promoter genetic variants may be related to sarcoidosis disease risk in AA.

Keywords

African-American race; apoptosis; DNA; Fas; genetic polymorphism; sarcoidosis; sequence analysis

Introduction

Sarcoidosis is a systemic granulomatous disease of unknown etiology that primarily affects the lung, but may affect any organ. Available evidence suggests that sarcoidosis occurs in individuals with a genetic predisposition exposed to an antigen that initiates a cell-mediated immune response. While the nature of this antigenic exposure remains unclear, we do know that recognition of antigen leads to accumulation of T lymphocytes and macrophages, and eventually granuloma formation. Available evidence supports the hypothesis that apoptosis

plays a role in the initiation and/or persistence of this immune response. Apoptosis, predominantly of macrophages within granulomas but also of lymphocytes, is observed in sarcoidosis biopsies; it is not apparent from normal lung parenchyma obtained from subjects undergoing lobectomy for a solitary pulmonary nodule (1). Increased sarcoidosis bronchoalveolar lavage (BAL) lymphocyte apoptosis and Fas ligand mRNA levels have also been observed, along with increased Fas expression on sarcoidosis BAL macrophages. A high correlation is found between sarcoidosis macrophage Fas expression and BAL lymphocyte percent, as well as other BAL indicators of active pulmonary sarcoidosis (1–4). The presence of apoptosis and Fas expression within granulomas and its association with markers of disease activity suggest a critical role for apoptosis in persistence of granulomatous inflammation. Findings in lung biopsies and BAL from patients with chronic beryllium disease, a granulomatous lung disease that is clinically and pathologically indistinguishable from sarcoidosis, also support a role for apoptosis in granulomatous inflammation (5–7).

The human Fas gene located on chromosome 10 contains a promoter region with numerous transcription factor binding sites and at least three confirmed promoter variants: G to A at position –1377, T to C at –690, and A to G at position –670 (8,9). The –670A Fas promoter polymorphism is located in an interferon-gamma-activated sequence (GAS), which is bound by transcription factors induced by a key lymphocyte cytokine, interferon gamma, (8). The GAS site is located within the sequence for the transcription factor STAT1 and is functional with the A allele, but abolished with the G variant. The –690 polymorphism is located in the enhancer region of the promoter, and despite its close physical proximity, does not appear to be in linkage disequilibrium with the –670 (8,9). The –1377 variant resides within a silencer region, with the A allele potentially altering the binding site of the transcription factor SP-1. The functional relevance of the –690 and –1377 variants has not been studied to date. Investigations of the relationship between the established –1377, –690 and –670 polymorphisms and inflammatory and autoimmune diseases have shown some associations, but with mixed results (9–12). Of note, several other single nucleotide polymorphisms (SNPs) in the promoter region of the *Fas* gene have been described (<http://snpper.chip.org/>); however, the three selected for this study are the only ones that have been confirmed by us and others in the published literature. No study to date has evaluated the association of Fas promoter polymorphisms with a granulomatous disease or with granulomatous disease severity.

Available evidence strongly supports a genetic contribution to the development of sarcoidosis (13,14). Several strong associations with specific human leukocyte antigen (HLA) alleles, other genes in the major histocompatibility complex region of chromosome 6 and elsewhere has been noted (13,15). It is likely that the genetic predisposition to sarcoidosis is multigenetic (13,14,16) and that much of the genetic attributable risk of this disease is yet to be determined. The strong evidence of a genetic predisposition to sarcoidosis, the support for an etiologic role of Fas-mediated apoptosis of immune cells in sarcoidosis and the existence of functional polymorphisms in the Fas promoter led us to design this study to address three questions. First, are there novel polymorphic sites in the Fas promoter? Second, is there an association between Fas polymorphisms, their extended haplotypes, and sarcoidosis? Finally, is there an association between these polymorphisms and haplotypes and sarcoidosis disease severity?

Materials and methods

Study populations and clinical assessment

Two distinct study populations were used in this study. Sequencing of the Fas promoter was performed on samples from 15 cases and 15 controls recruited at National Jewish Medical

and Research Center (NJMRC). Sarcoidosis cases were adults, aged 18–70, recruited from the Granuloma Clinic at NJMRC. All cases met the diagnostic criteria for sarcoidosis, established by the American Thoracic Society (ATS) consensus panel (17). Healthy control subjects, aged 18–70, were recruited from the hospital employee population.

Fas genotype and haplotype frequencies were examined in sarcoidosis subjects and controls from the National Institutes of Health (NIH) multicenter trial, A Case-control Etiologic Study of Sarcoidosis (ACCESS). A total of 706 incident sarcoidosis cases were enrolled within 6 months of diagnosis, and controls matched for age, sex, race and geography were recruited (18) from 10 clinical centers. The cases were characterized at the time of enrollment with a standardized assessment to determine disease status and organ involvement (19). Information regarding medication use at the time of enrollment, well-standardized spirometric measures and chest X-ray Scadding stages were also obtained (18). Longitudinal data were not available for the cohort. We used the spirometric data forced expiratory volume in 1 second and forced vital capacity (FEV1 and FVC), chest radiographic Scadding stage and use of systemic medications as surrogates for disease severity in the analysis as noted below. We used erythema nodosum as organ involvement indicating a better prognosis. We were unable to evaluate other organ involvement and its association with FAS genotypes because of small numbers (20). A total of 656 white and African-American (AA) pairs and case-control pairs were available for use in this study.

Informed consent was obtained from each study participant. The National Jewish Institutional Review Board approved the consents and the protocols.

Genomic DNA isolation

DNA was isolated from peripheral blood cells using the Wizard Genomic Purification Kit (Promega, Madison, WI). A similar method was used to isolate DNA from the ACCESS subjects.

DNA sequencing and analysis

We performed polymerase chain reaction (PCR) amplification and sequencing of six overlapping segments from position –1878 to +30 of the Fas promoter in 15 sarcoidosis cases and 15 healthy control subjects for a total of 30 subjects. We chose 30 subjects to help detect more common polymorphic variants (e.g. between 3% and 5%) vs rarer polymorphisms (<1%) as these variants would be likely to have greater attributable risk with a higher prevalence. Primer sequences are listed in Table 1. Sequences were amplified in 50 µl reactions containing approximately 1 µg of genomic DNA, 100 pM of each primer, 100 pM MgCl₂, 50 mM dNTPs and 4 U of Taq Gold polymerase as described by Welsh and Bunce (21). For the first and second segment, the conditions were (i) 94.0°C for 5.00 min, (ii) 94.0°C for 30 s, (iii) 60.1°C for 30 s, (iv) 72.0°C for 30 s, (v) Go to (ii), 39 times, (vi) 72.0°C for 7.00 min, (vii) 4.0°C forever. For the third through sixth segments, the cycling conditions were (i) 94.0°C for 5.00 min, (ii) 94.0°C for 30 s, (iii) 64.8°C for 30 s, (iv) 72.0°C for 30 s, (v) Go to (ii), 31 times, (vi) 72.0°C for 7.00 min, (vii) 4.0°C forever. PCR products were isolated using the Qiagen Gel Extraction Kit (Qiagen, Madison, WI).

The promoter segments were sequenced by Davis Sequencing (Davis, CA) using an automated ABI Prism 377 DNA sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA). The sequences were analyzed using AssemblyLIGN (Oxford Molecular Group PLC, Oxford, UK). Sites of single basepair misalignment were confirmed via examination of the electrophorogram using EditView (Perkin Elmer).

Genotyping via sequence-specific primer-polymerase chain reaction (SSP-PCR)

Genotyping for the Fas promoter polymorphisms is based on the methods of Welsh and Bunce (21). Briefly, combinations of allele-specific primers and consensus sequence primers are used to define genotypes for each of the Fas polymorphisms. Primers rely on dual specificity to provide a product size of 300–400 bp. The –1377, –690 and –670 primers are shown in Table 2; control primers were previously published (21). For quality control, sequence-specific primer-polymerase chain reaction (SSP-PCR) results were confirmed using sequenced samples. These sequenced samples were repeated randomly during the SSP-genotyping to ensure accurate genotyping of the homozygous common, heterozygous and homozygous rare genotypes. The SSP results were identical to those obtained by direct sequencing.

Determination of haplotypes

Haplotypes and posterior probabilities of correct assignment of haplotypes were determined using the Haplo.score program. This program uses an Expectation Maximization-based algorithm to calculate the posterior probability of each possible haplotype combination for each individual when haplotype phase is unknown. The Haploview program was used to calculate values for two measures of linkage disequilibrium, D' and r^2 , between polymorphic alleles in the evaluated haplotypes and to determine haplo-type blocks using the confidence interval method. D' is a measure of pairwise linkage disequilibrium that ranges between 0 and 1. $D' = 0$ represents complete linkage equilibrium (no correlation between snps), while $D' = 1$ represents complete linkage disequilibrium (no evidence for historical recombination). R^2 is also a measure of linkage disequilibrium in that it gives the statistical correlation of two snps while also taking into account the rarity of the polymorphisms.

Statistical analysis

Hardy–Weinberg equilibrium was assessed for each of the polymorphisms using chi-squared goodness-of-fit test. All analyses of the ACCESS population were stratified by race using a matched analysis. Analysis of genotype, allele and haplotype frequencies and disease status was performed using conditional logistic regression. First, an overall genotype test was performed to assess the most likely genetic model – dominant, additive or recessive – based on a comparison of effect sizes. Allele frequencies were then evaluated for their association with disease based on this ‘most likely’ genetic model – dominant, additive or recessive – based on a comparison of effect sizes. Second, allele frequencies were then evaluated for their association with disease or measures of disease severity based on the ‘most likely’ genetic model.

Analysis of genotype and allele frequencies and standard measures of disease severity available in the ACCESS database, including spirometric measures, chest radiographic Scadding stage and erythema nodosum, was performed using multiple linear and logistic regression. Spirometric data were used as percents predicted. Race-stratified univariate analyses were performed between the other three measures of severity and age and gender to determine if adjustment for these variables was necessary in the multivariable analysis, incorporating those variables with $P < 0.15$.

Haplotype frequencies in cases and controls were evaluated as noted above, comparing those having at least one copy of the haplotype of interest with those without the haplotype of interest as long as the conditional logistic regression equations converged. Haplotype associations with sarcoidosis were also evaluated in an unmatched fashion, incorporating the posterior probabilities of correct assignment of haplotype assigned by HAPLO. SCORE using a weighted logistic regression model (22). Results of the two haplotype analyses were compared and were consistent in all instances. The association between haplotype

frequencies and measures of disease severity was evaluated using multiple linear regression, comparing the effect of having at least one copy of the haplotype of interest with not having the haplotype of interest on each disease severity variable, adjusting for age and gender as determined above.

For all tests, a two-sided $P < 0.05$ was considered statistically significant. No correction for multiple comparisons was performed for allele or haplotype comparisons because of the highly correlated nature of the data. In cases where the tests being performed are correlated, traditional adjustments for multiple comparisons tend to be too conservative. We have chosen not to make these adjustments because this is an exploratory analysis. Instead of discounting the results, we hope that these findings will be followed-up and reproduced in other cohorts.

Results

Screening for novel Fas promoter polymorphisms in sarcoidosis cases and controls

Fas promoter sequencing was performed on 15 sarcoidosis cases and 15 controls to determine if there were any novel polymorphic sites in this region. These cases and controls were similar demographically, with a majority being female (10/15 cases; 12/15 controls), white (13/15 cases; 13/15 controls) and non-Hispanic (14/15 cases; 13/15 controls). Cases were significantly older than controls, with a mean age of 44 vs 37 years. Fas promoter sequencing confirmed the presence of three polymorphisms: -1377G to A, -690 T to C and -670A to G. No novel polymorphic sites were identified.

Significant race-based differences found in genotype distributions and haplotype associations with disease

A case-control study of Fas polymorphisms and sarcoidosis was undertaken using 656 matched case-control pairs from ACCESS. Of the 656 matched case-control pairs, 65% were female, 45% were AA and 55% were white. The median age of the cases enrolled in this study was 41 years (18–83 years) and of the controls was 41 years (19–79 years). About 94.4% of ACCESS cases had lung involvement at the time of enrollment, and 92% had abnormal chest radiographs (stages I–IV). Lung function abnormalities were less common: 34% had an abnormal FVC (<80% predicted), 40% had an abnormal FEV1 (<80% predicted) and 15% showed an FEV1/FVC ratio of <70%. Extrapulmonary involvement was noted in 49.7% of cases at baseline and was more common in AA than whites ($P < 0.001$).

Hardy-Weinberg equilibrium was confirmed in white and AA controls for the -1377 and -670 polymorphisms, and in AA controls for the -690. The -690 deviated from Hardy-Weinberg in white controls. As a result, we included the -690 polymorphism in our analyses of AA subjects. Allele and genotype distributions in whites for the -1377 and -670 were significantly different from those in AA (Table 3). All analyses to determine the association of these genotypes with disease were therefore stratified by race.

As this population was matched for gender and age, these variables were not included in the analysis. This analysis showed no significant associations between the -670 or -1377 polymorphisms and sarcoidosis in whites. In the AA, there was no association between the -670 or -1377 polymorphism and sarcoidosis. For the -690 polymorphism, there were no AA individuals who were homozygous for the rare C allele. Therefore, the only analysis that could be completed was based on an assumption of a dominant model, evaluating the presence of the rare allele (in heterozygotes) for an association with disease. There was a trend toward significance with a negative association of the -690C allele [odds ratio (OR) = 0.45; confidence interval (CI) (0.19–1.02), $P = 0.056$].

Haplotypes were then derived for -670 and -1377 polymorphisms in the white population and -670, -690 and -1377 polymorphisms in the AA population along with posterior probabilities of the likelihood of correct assignment of haplotype. Values for D' and r^2 for each pair of polymorphisms are summarized in Table 4, and the haplotype frequencies in cases and controls are shown in Table 5. No significant disease associations were noted with any Fas haplotype in whites (Tables 6 and 7). In AA, a trend toward a significant association was found between haplotype A1 and sarcoidosis [Tables 6 and 7; OR = 1.78, CI (1.0–3.2), $P = 0.05$]. The rarer haplotype, A4, was negatively associated with disease in AA [OR = 0.39 CI (0.16–0.93), $P = 0.03$]; this is consistent with the -690C allele association noted above.

Race-specific Fas genotype and haplotype associations with markers of sarcoidosis severity

Univariate analyses showed that in AA, gender was associated with erythema nodosum ($P = 0.05$) and steroid treatment ($P = 0.06$), and age was associated with steroid treatment ($P = 0.02$). Among whites, age was associated with erythema nodosum ($P = 0.06$). Therefore, age and/or gender were adjusted for in the genotype and haplotype analyses of association with steroid treatment and erythema nodosum as indicated by the univariate analyses.

Among white subjects, there were no statistically significant associations between -670 or -1377 genotypes and any markers of disease severity, including spirometric data (FEV1% and FVC%), presence/absence of erythema nodosum, chest radiograph Scadding stage and presence/absence of current steroid treatment (Table 8). Table 9 summarizes the genotype associations with markers of severity among AA, including a significant association between the -670 polymorphism and steroid use ($P = 0.01$). The analysis indicated an additive model, which yielded a cumulative OR, i.e. the overall risk associated with an incremental increase in the number of copies of the allele, of 1.68, CI (1.1–2.5). Among carriers of the A allele (heterozygotes and homozygotes), 61.8% reported current steroid use compared with 51.5% of those without an A allele (homozygous for the G allele).

Fas promoter polymorphism haplotypes were also evaluated with regard to their association with markers of disease severity (Table 10) and showed no significant associations among white sarcoidosis subjects. Among AA subjects, there was a significant negative association between haplotype A1 and steroid use (OR = 0.22 [CI (0.07–0.67)], $P = 0.008$). It is interesting to note that the -670A allele was associated with steroid use in the genotype analysis, and complementarily, containing the -670G allele was negatively associated with steroid use. The complementary nature of these findings supports that this promoter variant is associated more severe disease in AA with sarcoidosis.

Discussion

We confirmed the presence of the two most commonly evaluated sites at -670 and -1377 positions as well as a less commonly reported site at -690, but did not detect any novel polymorphisms. We evaluated these three polymorphisms in a large and well-characterized population of matched sarcoidosis–control pairs derived from ACCESS. Because of a deviation from Hardy–Weinberg in white controls for the -690 polymorphism, subsequent analysis was limited to the -670 and -1377 in this population; all three polymorphisms were evaluated in AA subjects. We found that Fas promoter polymorphism distributions vary by race and tend to be associated with sarcoidosis in AA. We further showed associations between these polymorphisms and a marker of disease severity (current steroid use) in AA.

Racial differences in genotype and allele distributions

Some of the most interesting aspects of this study were the clear differences between white and AA subjects, with regard to genotype distributions and unique associations with disease. These differences confirm the need to assess genotypes stratified by race, rather than assuming that similar results will be found in different racial groups. This is especially important in sarcoidosis, which disproportionately affects AA as compared with US whites, suggesting a unique genetic predisposition in this population. Most studies to date of disease association with Fas polymorphisms have focused on the -670 and -1377 (11,12,23) variants and have found genotype and allele distributions similar to our findings in whites. There are no previous reports in AA to compare our genotype and allele distributions.

The race-specificity of the genetic associations with disease in this study is not surprising and suggests that there may be racial associations with specific genes, possibly including Fas. Similarly, previous studies have shown that AA tend to be more severely affected by sarcoidosis, with higher rates of extrathoracic involvement (17,20). This difference in disease phenotype again suggests differences in underlying genetic predisposition to sarcoidosis that are specific to AA, possibly including Fas.

Several studies of sarcoidosis genetics also support the notion of race-specific genetic risk factors. Using the ACCESS population, Rossman et al. found different HLA alleles associated with sarcoidosis in AA and whites accounting for the highest population attributable risk in each group. Race-specific HLA associations were also noted with specific sites of organ involvement (24). The chromosomal regions associated with sarcoidosis in a genome-wide linkage study of white German sarcoidosis families were nearly completely distinct from those identified in a comparable study of AA sarcoidosis families (25,26). These findings support our study that racial differences in genetic predisposition occur in sarcoidosis disease risk and phenotype.

Association of Fas promoter polymorphisms with sarcoidosis

We found no associations between Fas promoter genotypes or haplotypes and sarcoidosis among whites. We did find two haplotype associations among AA: a trend toward a significant association with haplotype A1 (-1377G/-690T/-670G) and a negative association with haplotype A4 (-1377G/-690C/-670A). One possible explanation for these findings is that these haplotypes are each in linkage disequilibrium with different alleles at another polymorphic site(s), which either impacts Fas production or function and/or predisposition to sarcoidosis. It is also possible that one of the individual polymorphisms within the haplotype is driving the associations. As the haplotypes differ both in their -690 and their -670 alleles, either of these may be the relevant polymorphism. However, the lack of an association with the -670 genotypes, and the trend toward a significant negative association with the -690C allele with disease, would suggest that -690 is the more likely of the two. Although the D' between the -670 and -690 is 1.0, r^2 is low at 0.059, suggesting that the high D' is being driven by low rare allele frequencies and that there is no tight linkage disequilibrium between the two. We did not adjust for multiple comparisons because of the high correlation in the data. However, our findings of consistent results across multiple analysis of alleles and haplotypes and the consistency of the results support that these findings are not likely because of chance with multiple comparisons.

Association of Fas promoter polymorphisms with sarcoidosis severity

The selection of severity measures in sarcoidosis is complicated by multiorgan involvement and the lack of a single well-validated measure of global severity. We selected severity measures associated with prognosis and genetic factors in other studies, including chest radiograph Scadding stage and erythema nodosum (27,28). A strong and statistically

significant association was found between the -670A allele, which is the rare allele in this population, and more severe disease as indicated by current steroid use. As noted above, haplotype A1 was also positively associated with sarcoidosis; thus, it is possible that the disease association is being driven by a less severe sarcoidosis phenotype, which would be expected to also be negatively associated with steroid use. While we note that steroid use is an imperfect measure of severity, the compelling features of this association is the consistency of the statistical additive model (two A alleles conferring approximately twice the risk of one A allele) and the apparent functional importance of the A allele suggested by previous in vitro studies. Specifically, the -670 variant in the Fas gene promoter has been shown to influence gene expression, with the G variant abolishing a GAS-binding site within the sequence for the transcription factor STAT1. Plasmid constructs from a healthy subject homozygous for the A allele tended to show enhanced gene transcription compared with a construct from a subject homozygous for the G allele (11). Therefore, it is plausible that one A allele would result in increased Fas transcription and that two A alleles would result in even greater Fas transcription, which could affect macrophage and T-cell apoptosis and more severe disease. In fact, in a recent study, soluble Fas levels were associated with the -670 genotypes, with significantly higher soluble Fas levels noted in serum from subjects with the Fas -670 AA compared with those with the GG, supporting the increased transcription noted in the study above with the A allele (29). Unfortunately, we did not have serum or BAL fluid or cells available on the subjects enrolled in this study to assess the relationship between FAS genotypes and either soluble Fas or Fas on cells. However, a future study should determine the relationship between Fas genotypes and Fas expression.

Conclusions

The results of this study show some exciting genetic associations with sarcoidosis and sarcoidosis disease severity among AA. While the current state of knowledge of the functional relevance of these polymorphisms limits interpretation, the race specificity of the findings is intriguing. The genetic associations identified in this study may provide some insights into the susceptibility factors that influence the higher incidence and worse outcomes of sarcoidosis in AA. In fact, it seems likely that the more severe disease features in AA constitute a distinct phenotype with a distinct genetic predisposition. Given the results of this study, Fas polymorphisms may comprise an important component of this distinct genetic background.

Future studies should more comprehensively evaluate the Fas promoter for other potentially functional polymorphic sites. In addition, all these polymorphisms should be reevaluated in a distinct sarcoidosis and control population, including a more homogeneous white population, so that the -690 polymorphism could be evaluated in this group. Additional work is also needed to evaluate the functional importance of these polymorphisms in Fas production, and the importance of the Fas pathway and apoptosis in this form of granulomatous inflammation. Evaluation of genetic factors as predictors of disease severity will also require a cohort of sarcoidosis patients that can be followed longitudinally because this disease evolves over years, and the acuity and organ dysfunction of a patient's initial presentation may not reflect their long-term outcome.

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Table 1

Primers for sequenced segments of Fas promoter (all primers are written in 5' to 3' direction as they are found in GenBank sequences X87625 and D31968)

	5' primer	3' primer	Start site in <i>Fas</i> gene	End site in <i>Fas</i> gene
Segment 1	TATGGCATAGAAAGAGCAGGAC	GTGTCTATTAGATGCTCAGAGTGTG	-1878	-1395
Segment 2	TAATACAGAGAATGCCCATATACC	TCATTATCCAAACATACCTTCTGT	-1467	-974
Segment 3.2	AGCCTGAAGGATGAACAGTGG	GGCCAGGAAATAATGAGTAACGA	-1184	-839
Segment 3.4	GGCCAGGAAATAATGAGTAACGA	AGTGACTCCAGCAATTAGCCAAG	-861	-575
Segment 4	TTAACTGTCCATTCCAGGAACGTC	ATCTCGCGCAAGAGTGACACA	-688	-207
Segment 5	GAGGTCCTCACCTGAAGTGAGCAT	CATCTGGACCCTCCTACCTCTG	-457	+30

Table 2

Primers for Fas sequence-specific primer polymerase chain reaction (all primers are written in 5' to 3' direction)

	-1377 (rs2234767)	-690 (rs2234768)	-670 (rs1800682)
Polymorphic primer	TGCACAAGGCTGG CACA TGCACAAGGCTGG CACG	CTG GAA TGG ACA GTT AAC CA CTG GAA TGG ACA GTT AAC CG	TGG TTA ACT GTC CAT TCC AGG TGG TTA ACT GTC CAT TCC AGA
Consensus primer	AAG CTC TGC ACC TCA CTC TG	CTG CAT CCA AAT TCA GGT TCA	AAG GAG TCA GGG TTC GTT G
Direction of polymorphic primer	Sense	Antisense	Sense
Product size (bp)	316	355	322

Table 3

Allele and genotype frequencies by case–control status and race

	<u>White (n = 360/group)</u>		<u>African-American (n = 296/group)</u>	
	Case	Control	Case	Control
–1377G ^a	0.88	0.89	0.96	0.95
–1377A	0.12	0.11	0.04	0.05
–1377GG ^a	0.77	0.8	0.92	0.9
–1377GA	0.22	0.19	0.07	0.1
–1377AA	0.01	0.01	0.01	0
–690T	— ^b	—	0.99	0.97
–690C	—	—	0.01	0.03
–690TT	—	—	0.97	0.94
–690TC	—	—	0.03	0.06
–690CC	—	—	0	0
–670G ^a	0.44	0.44	0.75	0.70
–670A	0.56	0.56	0.25	0.30
–670GG ^a	0.19	0.18	0.56	0.49
–670GA	0.49	0.51	0.39	0.42
–670AA	0.32	0.31	0.05	0.09

^a Allele and genotype distributions differed significantly between white and African-American cases ($P < 0.0001$ for the –670 and –1377) and controls ($P < 0.0001$ for –670; $P < 0.001$ for –1377).

^b Genotype distributions of the –690 polymorphism among whites were not in Hardy–Weinberg equilibrium.

Table 4

Race-stratified measures of linkage disequilibrium between pairs of polymorphisms

	African-American (670–690)	African-American (670–1377)	African-American (690–1377)	White (670–1377)
D' (confidence interval)	1.0 (0.8–1.0)	0.82 (0.35–0.95)	0.37 (0.04–0.96)	1.0 (0.95–1.0)
r ²	0.059	0.012	0	0.163

Table 5

Haplotype map and frequencies^a for Fas promoter polymorphisms

	-1377	-690	-670	Frequency in cases	Frequency in controls
Whites					
W1	G	—	A	0.56	0.56
W2	G	—	G	0.32	0.33
W3	A	—	G	0.12	0.11
African-American					
A1	G	T	G	0.714	0.648
A2	G	T	A	0.23	0.268
A3	A	T	G	0.04	0.05
A4	G	C	A	0.01	0.03
A5	A	T	A	0.003	0.002
A6	A	C	A	0.003	0.003

^aHaplotype frequencies were calculated in the same fashion as allele frequencies except that the uncertainty associated with assignment of haplotype was included in the calculation (see Determination of haplotypes in the *Materials and methods*).

Table 6

Associations of Fas polymorphism genotypes with sarcoidosis

Fas polymorphism	Population	Overall genotype test heterozygote, OR (CI) ^a	Overall genotype test rare homozygote, OR (CI) ^a	Dominant model test, OR (CI) ^b	P value
-1377	White	1.2 (0.81-1.7)	0.78 (0.18-3.5)	1.2 (0.8-1.7)	0.45
-670	White	0.94 (0.68-1.3)	1.03 (0.68-1.6)	0.97 (0.7-1.3)	0.82
-1377	African-American	— ^c	— ^c	0.72 (0.4-1.3)	0.26
-690	African-American	— ^c	— ^c	0.45 (0.19-1.02)	0.056^d
-670	African-American	0.82 (0.59-1.1)	0.53 (0.28-1.02)	0.77 (0.56-1.1)	0.11

OR (CI), odds ratio (confidence interval).

^aThe comparison group for these analyses is the common homozygote, with an OR of 1.0.

^bBased on the overall genotype tests, all models in this analysis were dominant; therefore, this column represents the OR associated with carriage of the rare allele (heterozygote or homozygote; see Table 1 for allele frequencies).

^cOverall genotype test not performed and dominant model assumed secondary to no or very few individuals homozygous for the rare allele.

^dCorrected P value using Bonferroni correction is 0.17.

Statistically significant findings are in bold.

Table 7Associations of Fas polymorphism haplotypes with sarcoidosis^a

Fas haplotype	Population	OR (CI)	P value
W1 (-1377G/-670A)	White	0.94 (0.64–1.4)	0.77
W2 (-1377G/-670G)	White	1.04 (0.79–1.4)	0.78
W3 (-1377A/-670G)	White	1.15 (0.80–1.7)	0.45
A1 (-1377G/-690T/-670G)	African-American	1.78 (1.0–3.2)	0.05
A2 (-1377G/-690T/-670A)	African-American	0.84 (0.61–1.1)	0.27
A3 (-1377A/-690T/-670G)	African-American	0.70 (0.40–1.2)	0.21
A4 (-1377G/-690C/-670A)	African-American	0.39 (0.16–0.93)	0.03

OR (CI), odds ratio (confidence interval).

^a All analyses were performed using a dominant model, i.e. comparing individuals with one or two copies of the haplotype to those without the haplotype.

Statistically significant findings are in bold.

Table 8
Associations of Fas polymorphism genotypes with markers of sarcoidosis severity in whites

Fas polymorphism	Severity marker	Overall genotype test			Model	Model test, OR (CI) or parameter estimate (SE) ^b	P value
		Heterozygote, OR (CI) or parameter estimate (SE) ^a	Rare homozygote, OR (CI) or parameter estimate (SE) ^a	— ^c			
–1377	Erythema nodosum	— ^c	— ^c	— ^c	Dominant	2.15 (0.94–4.9)	0.07
–1377	Steroid use	0.70 (0.40–1.2)	3.6 (0.32–40.3)	— ^c	Additive	0.82 (0.50–1.4)	0.45
–1377	Scadding stage	0.64 (0.39–1.1)	1.9 (0.17–21.1)	— ^c	Additive	0.72 (0.45–1.15)	0.17
–1377	FEV1%	0.5 (2.1)	8.8 (9.4)	— ^c	Dominant	0.82 (2.1)	0.69
–1377	FVC%	–2.7 (2.1)	5.4 (9.6)	— ^c	Dominant	–2.4 (2.1)	0.26
–670	Erythema nodosum	1.5 (0.55–4.1)	2.5 (0.82–7.6)	— ^c	Additive	1.6 (0.9–2.8)	0.11
–670	Steroid use	0.81 (0.5–1.3)	0.76 (0.4–1.4)	— ^c	Dominant	0.8 (0.5–1.3)	0.33
–670	Scadding stage	0.97 (0.61–1.6)	1.6 (0.86–2.9)	— ^c	Additive	1.21 (0.9–1.6)	0.2
–670	FEV1%	3.8 (2.0)	–0.34 (2.5)	— ^c	Dominant	2.61 (1.9)	0.16
–670	FVC%	1.9 (2.0)	–2.2 (2.5)	— ^c	Dominant	0.76 (1.9)	0.69

OR (CI), odds ratio (confidence interval).

^aThe comparison group for these analyses is the common homozygote, with an OR of 1.0.

^bFor dominant models, this column represents the OR (dichotomous data) or parameter estimate (continuous data) associated with carriage of the rare allele (heterozygote or homozygote; see Table 1 for allele frequencies). For additive models, this column represents a cumulative OR, i.e. the overall risk associated with an incremental increase in the number of copies of the allele.

^cOverall genotype test not performed and dominant model assumed secondary to no or very few individuals homozygous for the rare allele in this severity category.

Table 9
Associations of Fas polymorphism genotypes with markers of sarcoidosis severity in African-Americans

Overall genotype test						
Fas polymorphism	Severity marker	Heterozygote, OR (CI) or parameter estimate (SE) ^a	Homozygote, OR (CI) or parameter estimate (SE) ^a	Model	Model test, OR (CI) or parameter estimate (SE) ^b	P value
−1377	Erythema nodosum	— _c	— _c	Dominant	1.08 (0.24–5.0)	0.92
−1377	Steroid use	— _c	— _c	Dominant	0.43 (0.18–1.04)	0.06
−1377	Scadding stage	— _c	— _c	Dominant	0.48 (0.2–1.14)	0.10
−1377	FEV1%	— _c	— _c	Dominant	2.6 (4.5)	0.56
−1377	FVC%	— _c	— _c	Dominant	2.5 (4.3)	0.56
−690	Erythema nodosum	— _c	— _c	Dominant	— _d	— _d
−690	Steroid use	— _c	— _c	Dominant	2.0 (0.39–10.1)	0.41
−690	Scadding stage	— _c	— _c	Dominant	0.25 (0.05–1.3)	0.10
−690	FEV1%	— _c	— _c	Dominant	0.25 (7.1)	0.97
−690	FVC%	— _c	— _c	Dominant	1.1 (6.8)	0.87
−670	Erythema nodosum	0.6 (0.24–1.5)	1.3 (0.27–6.4)	Additive	0.83 (0.41–1.7)	0.61
−670	Steroid use	1.4 (0.86–2.3)	5.2 (1.4–19.6)	Additive	1.68 (1.1–2.5)	0.01 ^e
−670	Scadding stage	0.68 (0.42–1.1)	1.14 (0.4–3.3)	Additive	0.84 (0.57–1.23)	0.36
−670	FEV1%	−1.2 (2.4)	−4.8 (5.2)	Dominant	−1.6 (2.3)	0.49
−670	FVC%	−1.4 (2.3)	−2.3 (4.9)	Dominant	−1.5 (2.2)	0.50

OR (CI), odds ratio (confidence interval).

^aThe comparison group for these analyses is the common homozygote, with an OR of 1.0.

^bFor dominant models, this column represents the OR (dichotomous data) or parameter estimate (continuous data) associated with carriage of the rare allele (heterozygote or homozygote; see Table 1 for allele frequencies). For additive models, this column represents a cumulative odds ratio, i.e. the overall risk associated with an incremental increase in the number of copies of the allele.

^cOverall genotype test not performed and dominant model assumed secondary to no or very few individuals homozygous for the rare allele; only one African-American case had genotype -1377AA and no African-American cases had genotype -690CC.

^dValidity of final model was questionable.

^eCorrected P value using Bonferroni correction is 0.03.

Statistically significant findings are in bold.

Table 10Associations of Fas polymorphism haplotypes with markers of sarcoidosis severity^a

Fas haplotype	Population	Severity marker	OR (CI) or parameter estimate (SE)	P value
W1 (−1377G/−670A)	White	Erythema nodosum	0.52 (0.22–1.3)	0.15
		Steroid use	1.2 (0.67–2.0)	0.59
		Scadding stage	0.63 (0.37–1.1)	0.08
		FEV1%	2.6 (2.2)	0.23
		FVC%	3.3 (2.2)	0.14
W2 (−1377G/−670G)	White	Erythema nodosum	1.7 (0.73–3.9)	0.22
		Steroid use	0.82 (0.53–1.3)	0.37
		Scadding stage	1.4 (0.93–2.2)	0.10
		FEV1%	1.3 (1.7)	0.45
		FVC%	0.97 (1.8)	0.58
W3 (−1377A/−670G)	White	Erythema nodosum	2.1 (0.94–4.9)	0.07
		Steroid use	0.75 (0.44–1.3)	0.29
		Scadding stage	0.67 (0.41–1.1)	0.11
		FEV1%	0.82 (2.1)	0.69
		FVC%	−2.4 (2.1)	0.26
A1 (−1377G/−690T/−670G)	African-American	Erythema nodosum	0.54 (0.15–2.0)	0.36
		Steroid use	0.22 (0.07–0.67)	0.008
		Scadding stage	1.1 (0.44–2.5)	0.89
		FEV1%	1.7 (4.4)	0.70
		FVC%	0.13 (4.2)	0.98
A2 (−1377G/−690T/−670A)	African-American	Erythema nodosum	0.75 (0.32–1.8)	0.51
		Steroid use	1.5 (0.96–2.5)	0.08
		Scadding stage	0.85 (0.54–1.4)	0.51
		FEV1%	−2.2 (2.4)	0.35
		FVC%	−1.9 (2.3)	0.39
A3 (−1377A/−690T/−670G)	African-American	Erythema nodosum	1.2 (0.25–5.4)	0.85
		Steroid use	0.46 (0.19–1.1)	0.09
		Scadding stage	0.52 (0.22–1.3)	0.15
		FEV1%	2.3 (4.6)	0.62
		FVC%	1.7 (4.4)	0.70
A4 (−1377G/−690C/−670A)	African-American	Erythema nodosum	__b	__b
		Steroid use	__b	__b
		Scadding stage	__b	__b
		FEV1%	__b	__b
		FVC%	__b	__b

OR (CI), odds ratio (confidence interval).

^a All analyses were performed with assumption of a dominant model, i.e. comparing individuals with one or two copies of the haplotype to those without the haplotype.

^b Analyses did not yield valid results secondary to small number of individuals with this haplotype.

Statistically significant findings are in bold.