Sarcoidosis and granuloma genes: a family-based study in African-Americans

B.A. Rybicki*, M.J. Maliarik*, L.M. Poisson*, M.C. Iannuzzi#

Sarcoidosis and granuloma genes: a family-based study in African-Americans. B.A. Rybicki, M.J. Maliarik, L.M. Poisson, M.C. Iannuzzi. ©ERS Journals Ltd 2004.
ABSTRACT: The evidence for a genetic component in the aetiology of sarcoidosis includes familial aggregation, associations with genetic polymorphisms, and linkage to the major histocompatibility complex class region on chromosome 6p. Unfortunately, the majority of genetic associations with sarcoidosis have not been consistently replicated.

In the present study, using a family-based study design, which controls for population stratification, the authors attempted to replicate previously reported associations between sarcoidosis and three attractive candidate genes studied primarily in case-control samples.

In 225 nuclear families, ascertained through African Americans with a history of sarcoidosis, no evidence was found for an association between sarcoidosis susceptibility and polymorphisms in the angiotensin converting enzyme, vitamin D receptor and tumour necrosis factor-α genes. Further analyses of chronic and acute disease phenotypes failed to reveal any notable associations. Assuming an underlying inheritance model with an additive allelic effect on disease risk, the current study had ∼80–90% statistical power to detect a 3-fold increased risk associated with the putative risk allele of the polymorphisms under study.

The present authors conclude that in African-Americans, the angiotensin converting enzyme, vitamin D receptor, and tumour necrosis factor-α genes are not significant risk factors for sarcoidosis susceptibility.
Eur Respir J 2004; 24: 251–257.

*Dept of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, Michigan and #Division of Pulmonary, Critical Care, and Sleep Medicine, Mount Sinai Medical Center, New York, New York, USA.

Correspondence: B.A. Rybicki, Dept of Biostatistics and Research Epidemiology, Henry Ford Health System, One Ford Place, 3E, Detroit, MI 48202, USA.
Fax: 1 3138746730
E-mail: brybick1@hfhs.org

Sarcoidosis is a systemic granulomatous disease of unknown aetiology that can affect any organ system, but most frequently includes the lungs (in ∼90% of cases), skin and eyes. A genetic susceptibility to sarcoidosis has been supported by studies demonstrating familial clustering [1] and a higher prevalence of disease in first-degree relatives [2]. Clustering of cases within specific ethnic groups also suggests a genetic susceptibility. In the USA, African Americans have a higher disease incidence and morbidity compared with Caucasians [3]. The pathological hallmark of sarcoidosis is multinucleated noncaseating granulomas in affected organs. Granuloma burden is indicative of disease activity and long-term active disease can lead to fibrosis and organ dysfunction. Key genes that play a role in granuloma formation and persistence include angiotensin converting enzyme (ACE), tumour necrosis factor (TNF)-α, and the vitamin D receptor (VDR).

The epithelioid cells in sarcoid-granulomas secrete >40 different cytokines and other mediators, including ACE [4]. Epithelioid cells of sarcoid granulomas contain ACE, and ACE synthesis in monocyte cell culture can be modulated by T-lymphocytes obtained from sarcoid patients [5]. Serum ACE levels in healthy individuals [6] and sarcoidosis patients [7] are influenced by a 287 base pair Alu sequence either present (insertion (I)) or absent (deletion (D)) in intron 16 of the ACE gene. Association studies of the ACE I/D polymorphism and sarcoidosis have produced mixed results. The ACE D allele, which is associated with higher serum ACE levels [6], has been found associated with increased sarcoidosis risk in African Americans [8], Japanese women [7] and in sarcoid-affected family members from a German population [9]. Conversely, other studies in both Caucasians [10, 11] and Japanese [12] have failed to find an association between the ACE I/D polymorphism and sarcoidosis susceptibility or progression.

TNF-α is a cytokine that probably has a key role in granuloma formation [13]. TNF-α is increased at sites of disease activity [14] and anti-TNF-α agents have shown promising therapeutic value in chronic sarcoidosis [15]. At least eleven polymorphisms in the promoter region of the TNF-α gene have been reported [16] but of these a G to A single base pair (bp) transition at position -307 is considered the most likely to affect gene transcription [16]. Several studies have examined this polymorphism in relation to sarcoidosis susceptibility [17–19], with a single case-control study in African Americans having null findings [20].

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 $(1,25(OH)_2D_3)$, a promoter of multinucleated giant cell formation [21], is produced at sites of sarcoid granulomas [22], and is elevated in sarcoidosis patients [23]. This hormone acts through the binding of intranuclear VDRs [24] and has been shown to interact directly with interferon gamma to promote the granulomatous process [25]. Several polymorphisms with potential functional significance have been identified in the VDR gene [26]. The Bsm I and Apa I polymorphisms are located in noncoding regions, whereas the *Taq* I polymorphism is in an exon but leads to a silent codon change with the nucleotides ATT and ATC both coding for

isoleucine at codon 352. The *b* allele of the Bsm I polymorphism, which has a reported association with sarcoidosis susceptibility [27], is in strong linkage disequilibrium with the absence of a *Taq* I cleavage site (*T* allele) in both Caucasians and African-Americans [28]. Another DNA sequence variant in the VDR gene is a thymine/cytosine (T/C) polymorphism in the first of two potential start (ATG) codons detected by restriction fragment length polymorphism using the endonuclease *Fok* I. The functional significance of the *Fok* I polymorphism is uncertain. However, most data indicate that the *F* allele, which lacks the first ATG start codon resulting in a transcribed protein three amino acids shorter than the variant *f* allele, is more effective in transactivation of the $1,25(OH)_2D_3$ signal [29]. One study has reported an association of the *f* allele with tuberculosis [30].

Since conflicting case-control reports of sarcoidosis associations with variants in the ACE, VDR and TNF-α genes exist, the present authors attempted to replicate these associations in a family-based study of sarcoidosis in African-American nuclear families. The strength of the family-based design is that it controls for genetic background unrelated to disease. While case-control samples are generally easier to collect and are statistically more powerful than family-based samples under some circumstances [31], case-control samples can produce spurious associations because of ethnic admixture or other problems relating to inappropriate controls [32]. Therefore, the results presented here should help clarify the role of the ACE (I/D), VDR (*Taq*I and *Fok* I) and TNF-α (-307) genetic polymorphisms with regard to sarcoidosis risk in African Americans.

Methods

Study sample

The study protocol was approved by the Henry Ford Hospital Institutional Review Board. Diagnosis was confirmed by tissue biopsy in 86% of the index cases and in all cases with normal (stage 0) chest radiographs. The cases without histological confirmation had radiographical evidence of bilateral hilar adenopathy, a compatible clinical presentation and were observed for ≥2 yrs with no other medical condition that could explain the clinical course.

From a total of 623 eligible probands, 359 (58%) were enrolled with one or more first-degree family member(s). Of these, 234 had two or more parents or sibs that donated a blood sample. An additional 10 African-American sarcoidosis families were recruited outside the Henry Ford Health System, resulting in 244 total families. Both parents were genotyped when available. When one or both parents were unavailable for genotyping, all available full sibs were genotyped. Using a panel of unlinked markers, 20 families (8.2%) with Mendelian segregation inconsistencies and two families (0.8%), where the proband, after further follow-up, was not found to have sarcoidosis, were excluded from the analysis. Three of the remaining 222 families had two separate nuclear families, which were analysed separately, resulting in 225 nuclear families or a total of 704 individuals for study. The three basic family configurations are shown in table 1. While the majority of family members with a sarcoidosis history were probands through whom the family was ascertained, there were also 22 sibs and four additional family members (who were treated as index cases in the analysis) with a history of sarcoidosis based on self-report of a physician diagnosis of this condition. In 18 of the 22 sib cases, and the other four additional family member cases, the diagnosis was confirmed by biopsy. In the remaining four sib cases, a medical record

Table 1. – Breakdown of the different family configurations of the 225 African-American sarcoidosis nuclear families in the study population

	Number of affected offspring	
	1	2+
Family configurations		
Both parents	43 (19.1)	2 (0.9)
1 parent, ≥2 offspring	97 (43.1)	7 (3.1)
No parents, ≥3 offspring	66 (29.3)	10 (4.4)

Data are presented as n (% total).

review revealed a chest radiograph report of bilateral hilar adenopathy at the time of diagnosis. Most families (90%) had a single affected offspring and >80% of families had additional unaffected sibs.

Phenotyping

Using a previously described criteria used for organ system involvement [33], affected sibs were phenotypically categorised under three broad categories: mild, moderate, or severe. Mild cases were asymptomatic or had normal organ systems without treatment, or spontaneously resolved to normal within 2 yrs of presentation. Moderate cases had a symptomatic disease course with minimal residual abnormality in any organ with or without treatment, or a disease course for >3 yrs with some residual abnormality despite therapy. Severe cases had progressive organ dysfunction for ≥3 yrs, despite therapy.

Genotyping

High molecular weight DNA was isolated from anticoagulated blood by detergent lysis and organic extraction. The diallelic ACE polymorphism was genotyped by enzymatic amplification of a segment within intron 16 with the 190 and 490 base pair alleles resolved by 6% polyacrylamide gel electrophoresis. The primers were designed as previously described [8]. Amplification was performed using 100 ng of genomic DNA and 10 picomol (pmol) of each primer at 94°C for 1 min, 58°C for 1 min and 72°C for 2 min, for 30 cycles. To confirm that genotypes with only a D band were true homozygotes rather than mistyped ID heterozygotes due to preferential amplification of the D allele, a second reaction was performed using the sense primer labelled with gamma [^{32}P] ATP. Alleles were separated by acrylamide gel electrophoresis, stained with ethidium bromide to display molecular weight markers and autoradiographed to detect alleles.

The VDR *Taq* I and *Fok* I polymorphisms were typed by amplification and restriction fragment length polymorphism as previously described [30]. *Taq* I identifies a silent T to C base change at nucleotide 352. *Fok* I identifies a C to T transition that creates an alternative initiation site three codons upstream from a second ATG start site. Alleles were separated by agarose gel electrophoresis, and detected with ethidium bromide. Each DNA sample was assayed at least twice per marker, to confirm genotype.

The G to A transition at position -307 of the TNF-α gene was determined by amplification of a 413 bp region incorporating two polymorphisms. Amplification was performed using the sense primer 5′-TTCCTGCATCCTGTCTGGAA-3′ and antisense primer, 5′-AGGGAGCGTCTGA-TGGCTG-3′ at 1 pmol per reaction, 2 mM deoxynucleotidetriphosphates, 10 ng DNA, and 0.62 unit *Taq* DNA polymerase (Promega,

Table 2. – Association between the angiotensin converting enzyme (ACE) insertion (I)/deletion (D), vitamin D receptor (VDR) *Fok* I and *Taq* I, and tumour necrosis factor (TNF)-307 polymorphisms and sarcoidosis susceptibility in African-Americans families based on a multiallelic test using the Family-Based Association Test software

Polymorphism	Dominant model[#]		Additive model[¶]	
	Chi-squared test	p-value	Chi-squared test	p-value
ACE I/D	2.22	0.33	0.06	0.81
VDR *Fok* I	0.89	0.64	0.01	0.91
VDR *Taq* I	0.02	0.99	<0.01	0.95
TNF-307	0.34	0.84	<0.01	0.98

[#]: 2 degrees of freedom; [¶]: 1 degree of freedom.

Madison, WI, USA). Amplification conditions were 94°C for 60 s, 62°C for 60 s, 72°C for 60 s, with a final extension step of 6 min at 72°C. Products were sequenced in both directions on a Beckman CEQ 2000XL, by the CEQ Dye Terminator Cycle Sequencing kit, according to the manufacturer′s protocol (Beckman Coulter, Fullerton, CA, USA). All genotypes were performed in duplicate with genotypes in disagreement (~10%) run a third time to verify results.

Statistical methodology

To determine whether one or more alleles at the locus of interest was associated with the sarcoidosis phenotype, a Family-Based Association Test (FBAT) statistic, S, calculated using the FBAT software was used [34]. In short, this test statistic determines whether a certain allele in a nuclear family is transmitted to affected offspring more (or less) often than would be expected under Mendelian segregation. Test statistics for both dominant and additive inheritance models were computed. The outcome for initial analyses was a dichotomous affection status variable. More in depth phenotype models were also examined in which the three phenotypic levels were treated as either a dichotomous or an ordinal variable. For the former parameterisation, a multivariate model was used where three 0, 1 dummy variables were coded corresponding to absence or presence of the phenotype in question. For the latter parameterisation, affected sibs were coded as 1, 2 or 3 with a higher number corresponding to a more severe phenotypic level. For both types of parameterisations, unaffected sibs were coded as zero.

The statistical power of the study sample for detecting an association between the putative risk allele of each polymorphism and sarcoidosis was calculated with the power calculation of Family-Based Test software [35]. Power calculations were conditional on the frequency of each risk allele, an estimated lifetime risk of 1% [3], the study sample configuration depicted in table 1 and a range of genotype relative risks (γs). Using these parameters, expected genotypic-specific penetrance values, f_2 (two risk alleles), f_1 (one risk allele), and f_0 (no risk alleles), under additive ($f_0=2f_1-f_2$), multiplicative ($f_0=f_1^2/f_2$) and dominant ($f_1=f_2$) inheritance models could be calculated. The routine used to compute power estimates for the family-based analysis was based on a second-order Taylor expansion, which has been shown to have good precision for samples sizes of $\geqslant 100$ families [35].

Results

Multiallelic test statistics for the four polymorphisms tested, ACE I/D, VDR *Fok* I and *Taq* I, and TNF-307 are shown in table 2. Statistical p-values did not reach significance (p=0.05) for either dominant or additive models of inheritance. Table 3 shows the biallelic association tests for the putative risk allele of each polymorphism. In table 3, both the observed and expected values of allelic transmissions, S and E(S), respectively, are based on the genetic model postulated. Computation of the variance of S, allowed estimation of a significance level for a large number of families based on a Z statistic, which is normally distributed with mean 0 and variance 1, and defined as:

$$Z = [S - E(S)]/\sqrt{\text{Variance(S)}} \quad (1)$$

Under both dominant and additive models of inheritance, none of the four alleles markedly deviated from the null hypothesis. Since it is possible that the polymorphisms under study could be risk factors for disease progression rather than susceptibility, the outcome of disease phenotype was analysed next instead of affection status. Phenotype data were available for sibs in 163 of the 225 (72%) study families. Table 4 depicts four different types of analyses based on inheritance models (dominant and additive) and phenotypic parameterisations. While no statistically significant results were observed, the VDR *Fok* I polymorphism had a marginally significant result (p=0.08) under an additive model with a multivariate phenotype. For the dichotomous phenotypic outcome, no assumptions were made as to the relationship of one phenotypic category with another, as compared with the

Table 3. – Association between selected alleles of the angiotensin converting enzyme (ACE) insertion (I)/deletion (D), vitamin D receptor (VDR) *Fok* I and *Taq* I and tumour necrosis factor (TNF)-307 polymorphisms and sarcoidosis susceptibility in African-Americans families based on a biallelic test using the Family-Based Association Test software

Polymorphism*allele	Allele frequency %	Ninf	S	E(S)	S/E(S)	Variance (S)	Z score	p-value
Dominant model								
ACE I/D*D	58.7	51	105	108.1	0.97	12.9	-0.85	0.39
VDR *Fok* I*f	23.9	75	62	59.7	1.04	18.3	0.53	0.60
VDR *Taq* I*t	27.2	100	70	70.6	0.99	24.9	-0.11	0.91
TNF-307*A	14.3	44	35	35.9	0.97	10.7	-0.26	0.79
Additive model								
ACE I/D*D	58.7	116	160	158.5	1.01	43.3	0.24	0.81
VDR *Fok* I*f	23.9	88	73	72.4	1.01	27.6	0.11	0.91
VDR *Taq* I*t	27.2	109	80	80.4	1.00	33.2	-0.06	0.95
TNF-307*A	14.3	56	45	44.9	1.00	16.4	0.02	0.98

Data are presented as n unless otherwise stated. Ninf: number of informative families; S: number of observed allelic transmissions; E(S): expected number of allelic transmissions under stated model of inheritance. Both observed and expected values of allelic transmissions, S and E(S), respectively, are based on the genetic model postulated. Computation of the variance of S, allowed estimation of a significance level for a large number of families based on a Z statistic, defined as $Z=[S-E(S)]/\sqrt{\text{variance (S)}}$, which is normally distributed with mean 0 and variance 1.

Table 4. – Association between the angiotensin converting enzyme (ACE) insertion (I)/deletion (D), vitamin D receptor (VDR) *Fok* I and *Taq* I, tumour necrosis factor (TNF)-307 and polymorphisms and sarcoidosis disease severity in African-American families based on a multiallelic test using the Family-Based Association Test software

Polymorphism	Dichotomous phenotype[#]		Ordinal phenotype[¶]	
	Chi-squared (dof)	p-value	Chi-squared (dof)	p-value
Dominant model				
ACE I/D	4.71 (5)	0.45	3.48 (2)	0.18
VDR *Fok* I	5.59 (3)	0.13	3.64 (2)	0.16
VDR *Taq* I	0.74 (3)	0.86	0.28 (2)	0.87
TNF-307	1.19 (2)	0.55	0.50 (2)	0.78
Additive model				
ACE I/D	0.35 (3)	0.95	0.35 (1)	0.62
VDR *Fok* I	5.06 (2)	0.08	0.06 (1)	0.80
VDR *Taq* I	1.02 (3)	0.80	0.01 (1)	0.93
TNF-307	0.64 (2)	0.73	0.08 (1)	0.78

Data are presented as Chi-squared values unless otherwise stated. dof: degrees of freedom. [#]: The dichotomous phenotype was parameterised 1, 0 in a four-level multivariate model; [¶]: The ordinal phenotype was parameterised as 0, 1, 2 and 3 with each increasing level representing a greater level of disease severity.

ordinal phenotypic outcome that was modelled such that increasingly severe phenotypic categories had higher covariate values. Among the phenotyped-affected sibs, the VDR *Fok**f allele had an increasingly higher frequency by severity of phenotypic category, *i.e.* 33.8% in mild cases, 42.5% in moderate cases and 51.7% in severe cases. The frequency of the VDR *Fok**f allele among the unaffected sibs in these families was 41.3%.

Since the presented results were largely negative, the current authors next tested what statistical power the present study sampled afforded. Statistical power was calculated for the putative risk allele of each of the four polymorphisms under study. For each allele and inheritance model combination, genotype relative risks (γ) ranging 1–5 were tested at the type I error (α) levels of 5 and 1% (fig. 1). For the ACE I/D*D allele, γs of $\geqslant 3$ were detected at the α=0.05 level with ~80% statistical power under an additive inheritance model, and with 90% statistical power under a multiplicative inheritance model. At the α=0.01 level for the additive and multiplicative models, γs of $\geqslant 4$ could be detected with $\geqslant 80\%$ power depending on the inheritance model. Under a dominant inheritance model, the statistical power only approached 60% for a γ value of 5 at α=0.05. For the VDR *Fok* I*f and *Taq* I*t alleles, γs of $\geqslant 3$ were detected at the α=0.05 level with $\geqslant 90\%$ statistical power under all three inheritance models tested. Likewise γs of $\geqslant 3$ could be detected with $\geqslant 70\%$ statistical power under all three inheritance models tested at the α=0.01 level. Statistical power for γs of $\geqslant 4$ could be detected with ~100% power under all three inheritance models tested at both α levels (0.01 and 0.05) tested. Statistical power estimates for the TNF-307*A allele were virtually identical to the two VDR alleles under the dominant and additive inheritance models and slightly less under the multiplicative inheritance model.

Discussion

Noncaseating granulomas are the histological hallmark of sarcoidosis, therefore, the present study investigated polymorphisms in candidate genes probably involved in granuloma formation and maintenance. Conflicting case-control study results with respect to sarcoidosis risk exist for polymorphisms in the ACE [7–12, 36–40], VDR [27, 41], and TNF-α [17–20, 42] genes (table 5). Therefore, the current authors tested polymorphisms in these genes for associations with sarcoidosis in African-American families. Although the present study had sufficient statistical power to detect effect sizes of $\geqslant 3$ under an additive inheritance model, no significant associations were found.

One possible reason for the inconsistent results across sarcoidosis genetic association studies could be case heterogeneity. Inclusion of misdiagnosed cases will bias results towards the null hypothesis, but an unrepresentative case group can either artificially increase or decrease an odds ratio. Many sarcoidosis studies rely on cases drawn from specialised clinics that treat more severe and advanced cases. If any of these polymorphisms are truly related to sarcoidosis progression or disease subtypes, as several studies report for the ACE [8, 36] and TNF-α [17, 42] polymorphisms, then a disproportionate mix of disease types among cases may bias an odds ratio measuring sarcoidosis susceptibility. In the present study population most cases were phenotyped, so the present authors were able to examine the relationship between the four polymorphisms under study and disease severity. No association was found between either the ACE I/D or TNF-307 polymorphisms and disease phenotype, which would suggest that neither of these polymorphisms are related to disease progression in African Americans and that the case mix in the present study population did not influence its susceptibility results. If the TNF-307 polymorphism is actually a marker for Lofgrens syndrome and erythema nodosum in sarcoidosis, as several studies report [17, 42], then these conditions being rare in African-American sarcoidosis cases [43] may explain the negative findings with respect to this polymorphism in the current study. However, the extent to which the current authors could exclude a disease progression gene was limited by a smaller sample size due to missing and incomplete phenotype data, which did not allow the present study to consider the full range of phenotypic expressions the genes under study could exhibit.

Genetic and environmental background can also affect case-control genetic association results. All four polymorphisms that were studied are observed in Caucasian, Japanese and African-American populations but their effect on disease susceptibility may be altered by other genetic or environmental factors. If these factors vary among ethnic groups, then ethnic-specific associations of a given polymorphism with sarcoidosis susceptibility are possible.

In the study by NIIMI *et al.* [27], the Bsm I *B* allele was overrepresented in sarcoidosis patients but GULEVA and SEITZER [41] subsequently reported a failure to replicate this finding in a German Caucasian population. In the current study population, the corresponding *t* allele for the *Taq* I
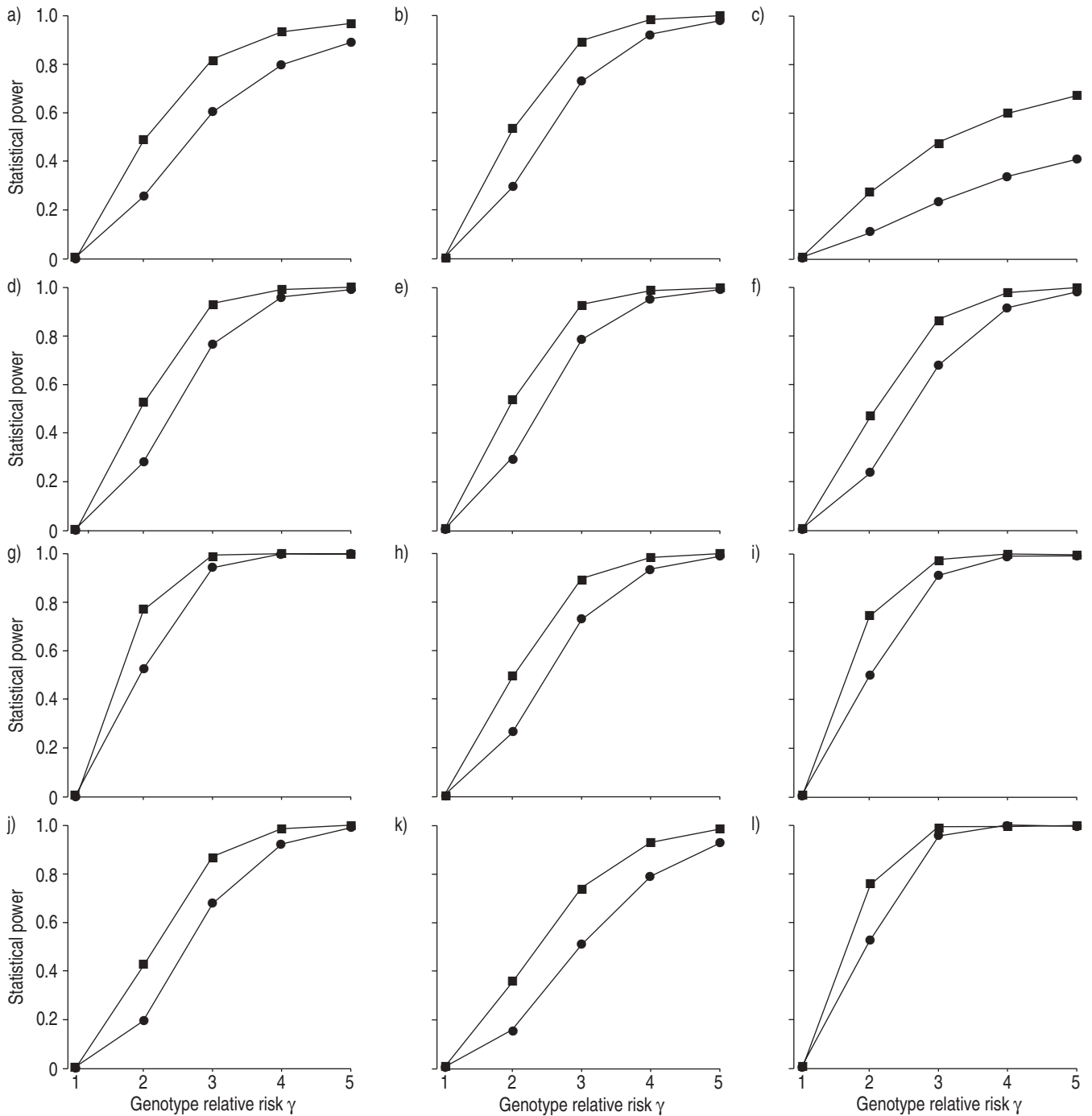
Fig. 1. – Statistical power estimates for the angiotensin converting enzyme (ACE) insertion (I)/ deletion (D)*D allele (a–c), vitamin D receptor (VDR) *Fok* I*f (d–f), VDR *Taq* I*t (g–i) and tumour necrosis factor (TNF)-307*A (j–l) alleles to detect genotype relative risks (γ) ranging 1–5 under additive (a, d, g and j), multiplicative (b, e, h and k) and dominant inheritance (c, f, i and l) models and type I error levels of 0.05 (■) and 0.01 (●).

polymorphism showed no preferential transmission to affected offspring. The current study also examined the *Fok* I VDR polymorphism, which is in linkage equilibrium with the *Taq* I/Bsm I allele group [30], and found that this polymorphism was not associated with sarcoidosis susceptibility but the variant *f* allele may have a modest association with chronic sarcoidosis. The potential functional relevance of the *Fok* I polymorphism [29] and the association of VDR genotypes with other granulomatous disorders [44, 45] would suggest that the VDR gene warrants further investigation in sarcoidosis. However, in terms of sarcoidosis susceptibility

both VDR alleles tested in the present study had an 80% power to detect a 2-fold increased risk under a dominant model, which is the risk level for the heterozygote VDR Bsm I polymorphism previously reported [27].

The extent to which population stratification biases results of case-control genetic association studies is a debated topic. While in theory, selection of a control sample from the same population that cases are drawn should avoid population stratification, in practice uniform case/control sampling is difficult to achieve. Statistical methods to adjust for population stratification using population-specific alleles

Table 5. – Summary of case-control studies of sarcoidosis susceptibility in relation to angiotensin converting enzyme (ACE) insertion (I)/deletion (D), tumour necrosis factor (TNF)-307 and vitamin D receptor (VDR) gene polymorphisms

Polymorphism [reference]	Population	Risk allele#	Cases/controls	ORHet (95% CI)	ORHom (95% CI)
ACE (I/D)					
ARBUSTINI [11]	Italian Whites	0.60	61/80	0.54 (0.20–1.49)	1.03 (0.37–2.91)
FURUYA [7]	Japanese	0.33	103/341	1.69 (1.04–2.73)	1.37 (0.68–2.75)
GARRIB [37]	UK Whites	0.47	54/100	2.38 (0.88–6.44)	4.50 (1.53–13.25)
MALIARIK [8]	US Whites	0.58	60/49	0.66 (0.23–1.90)	0.50 (0.16–1.61)
	US Blacks	0.44	183/111	1.30 (0.72–2.36)	3.17 (1.50–6.71)
MCGRATH [10]	UK Whites	0.54	180/386	0.92 (0.59–1.44)	0.86 (0.53–1.40)
	Czech Whites	0.47	56/179	0.74 (0.36–1.49)	1.02 (0.45–2.33)
PAPADOPOULOS [38]	Swedish Whites	0.52	62/107	0.51 (0.23–1.11)	0.91 (0.39–2.12)
PIETINALHO [36]	Finnish	0.51	59/70	1.78 (0.69–4.56)	1.79 (0.64–5.03)
SHARMA [39]	UK Whites	0.52	47/146	1.36 (0.58–3.18)	1.33 (0.54–3.30)
TAKEMOTO [40]	Japanese	0.37	100/247	1.44 (0.87–2.38)	0.80 (0.38–1.69)
TOMITA [12]	Japanese	0.35	207/314	1.28 (0.88–1.88)	1.31 (0.75–2.29)
TNF-307					
GRUTTERS [17]	UK Whites	0.19	96/354	1.10 (0.68–1.80)	1.28 (0.40–4.10)
	Dutch Whites	0.21	100/222	0.89 (0.54–1.49)	0.63 (0.17–2.37)
PANDEY [20]	US Whites	0.17	278/278	1.16 (0.81–1.68)	1.32 (0.51–3.42)
	US Blacks	0.14	219/219	1.11 (0.72–1.71)	0.25 (0.03–2.29)
SEITZER [19]	German Caucasians	0.19	101/216	1.13 (0.67–1.92)	1.85 (0.70–4.90)
YAMAGUCHI [18]	Japanese	0.01	110/161	1.47 (0.20–10.61)	1.47 (0.20–74.76)
VDR					
NIIMI [27]	Japanese	0.11	101/105	2.42 (1.29–4.53)	1.34 (0.08–21.82)
GULEVA [41]	European	0.32	85/80	0.86 (0.45–1.64)	0.72 (0.25–2.27)

Data are presented as n unless otherwise stated. OR: Odds ratio; CI: confidence interval. #: frequency in controls; ORHet (95% CI): OR and 95% CI for heterozygote genotype with risk allele; ORhom (95% CI): OR and 95% CI for homozygote genotype with risk allele.

have been proposed [46] but these methods have yet to be applied widely and may be prone to overcorrection of potential biases. The family-based study design used in the present study provides a sampling procedure that avoids the potential bias of case-control sampling without making any elaborate statistical corrections.

In summary, the present study found no evidence for an association between sarcoidosis susceptibility in African Americans and three key genes, angiotensin converting enzyme, vitamin D receptor and tumour necrosis factor-α, involved in granuloma formation. Overall results from past case-control studies of polymorphisms in these genes are equivocal with regard to sarcoidosis susceptibility. The results of the present study should not be subject to population stratification since potential confounding by genetic background was controlled in the study design. The current study was also sufficiently powered to detect levels of association with disease susceptibility that have been previously reported. The presented results suggest that genetic control of the granulomatous reaction is not related to sarcoidosis susceptibility in African Americans. Alternatively, the angiotensin converting enzyme, vitamin D receptor and tumour necrosis factor-α genes may not be optimal candidates for studying the sarcoidosis granulomatous reaction in relation to disease susceptibility in African Americans.

References

1. Rybicki BA, Iannuzzi MC, Frederick MM, et al. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). *Am J Respir Crit Care Med* 2001; 164: 2085–2091.

2. Rybicki BA, Kirkey KL, Major M, et al. Familial risk ratio of sarcoidosis in African-American sibs and parents. *Am J Epidemiol* 2001; 153: 188–193.

3. Rybicki BA, Major M, Popovich JJ, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. *Am J Epidemiol* 1997; 145: 234–241.

4. Silverstein E, Friedland J, Lyons HA, Gourin A. Markedly elevated angiotensin converting enzyme in lymph nodes containing non-necrotizing granulomas in sarcoidosis. *Proc Natl Acad Sci USA* 1976; 73: 2137–2141.

5. Silverstein E, Pertschuck LP, Freiedland J. Immunofluorescent localization of angiotensin converting enzyme in epithelioid and giant cells of sarcoidosis granuloma. *Proc Natl Acad Sci USA* 1979; 76: 6646–6648.

6. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphisim in the angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86: 1343–1346.

7. Furuya K, Yamaguchi E, Itoh A, et al. Deletion polymorphism in the angiotensin I converting enzyme (ACE) gene as a genetic risk factor for sarcoidosis. *Thorax* 1996; 51: 777–780.

8. Maliarik MJ, Rybicki BA, Malvitz E, et al. Angiotensin-converting enzyme gene polymorphism and risk of sarcoidosis. *Am J Respir Crit Care Med* 1998; 158: 1566–1570.

9. Schurmann M, Reichel P, Muller-Myhsok B, et al. Angiotensin-converting enzyme (ACE) gene polymorphisms and familial occurrence of sarcoidosis. *J Intern Med* 2001; 249: 77–83.

10. McGrath DS, Foley PJ, Petrek M, et al. Ace gene I/D polymorphism and sarcoidosis pulmonary disease severity. *Am J Respir Crit Care Med* 2001; 164: 197–201.

11. Arbustini E, Grasso M, Leo G, et al. Polymorphism of angiotensin-converting enzyme gene in sarcoidosis. *Am J Respir Crit Care Med* 1996; 153: 851–854.

12. Tomita H, Ina Y, Sugiura Y, et al. Polymorphism in the angiotensin-converting enzyme (ACE) gene and sarcoidosis. *Am J Respir Crit Care Med* 1997; 156: 255–259.

13. Shikama Y, Kobayashi K, Kasahara K, *et al.* Granuloma formation by artificial microparticles in vitro. Macrophages and monokines play a critical role in granuloma formation. *Am J Pathol* 1989; 134: 1189–1199.

14. Kaneshima H, Nagai S, Shimoji T, *et al.* TNF alpha mRNA, but not IL-1 beta, is differentially expressed in lung macrophages of patients with active pulmonary sarcoidosis. *Sarcoidosis* 1994; 11: 19–25.

15. Baughman RP, Iannuzzi M. Tumour necrosis factor in sarcoidosis and its potential for targeted therapy. *BioDrugs* 2003; 17: 425–431.

16. Allen RD. Polymorphism of the human TNF-alpha promoter-random variation or functional diversity? *Mol Immunol* 1999; 36: 1017–1027.

17. Grutters JC, Sato H, Pantelidis P, *et al.* Increased frequency of the uncommon tumor necrosis factor -857T allele in British and Dutch patients with sarcoidosis. *Am J Respir Crit Care Med* 2002; 165: 1119–1124.

18. Yamaguchi E, Itoh A, Hizawa N, Kawakami Y. The gene polymorphism of tumor necrosis factor-beta but not that of tumor necrosis factor-alpha, is associated with the prognosis of sarcoidosis. *Chest* 2001; 119: 753–761.

19. Seitzer U, Swider C, Stuber F, *et al.* Tumour necrosis factor alpha promoter gene polymorphism in sarcoidosis. *Cytokine* 1997; 9: 787–790.

20. Pandey JP, Frederick M. TNF-alpha, IL1-beta, and immunoglobulin (GM and KM) gene polymorphisms in sarcoidosis. *Hum Immunol* 2002; 63: 485–491.

21. Ohta M, Okabe T, Ozawa K, Urabe A, Takaku F. In vitro formation of macrophage-epithelioid cells and multinucleated giant cells by 1 alpha, 25-dihydroxyvitamin D3 from human circulating monocytes. *Ann N Y Acad Sci* 1986; 465: 211–220.

22. Sharma OP. Vitamin D, calcium, and sarcoidosis. *Chest* 1996; 109: 535–539.

23. Bell NH, Stern PH, Pantzer E, Sinha TK, DeLuca HF. Evidence that increased circulating 1 alpha, 25-dihydroxyvitamin D is the probable cause for abnormal calcium metabolism in sarcoidosis. *J Clin Invest* 1979; 64: 218–225.

24. Malloy PJ, Pike JW, Feldman D. The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. *Endocr Rev* 1999; 20: 156–188.

25. Vidal M, Ramana CV, Dusso AS. Stat1-vitamin D receptor interactions antagonize 1, 25-dihydroxyvitamin D transcriptional activity and enhance stat1-mediated transcription. *Mol Cell Biol* 2002; 22: 2777–2787.

26. Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. *Epidemiol Rev* 2000; 22: 203–217.

27. Niimi T, Tomita H, Sato S, *et al.* Vitamin D receptor gene polymorphism in patients with sarcoidosis. *Am J Respir Crit Care Med* 1999; 160: 1107–1109.

28. Bell NH, Morrison NA, Nguyen TV, Eisman J, Hollis BW. ApaI polymorphisms of the vitamin D receptor predict bone density of the lumbar spine and not racial difference in bone density in young men. *J Lab Clin Med* 2001; 137: 133–140.

29. Whitfield GK, Remus LS, Jurutka PW, *et al.* Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Mol Cell Endocrinol* 2001; 177: 145–159.

30. Wilkinson RJ, Llewelyn M, Toossi Z, *et al.* Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet* 2000; 355: 618–621.

31. Schaid DJ, Rowland C. Use of parents, sibs, and unrelated controls for detection of associations between genetic markers and disease. *Am J Hum Genet* 1998; 63: 1492–1506.

32. Freedman ML, Reich D, Penney KL, *et al.* Assessing the impact of population stratification on genetic association studies. *Nat Genet* 2004; 36: 388–393.

33. Judson MA, Baughman RP, Teirstein AS, Terrin ML, Yeager H, Jr. Defining organ involvement in sarcoidosis: the ACCESS proposed instrument. ACCESS Research Group. A case control etiologic study of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999; 16: 75–86.

34. Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. *Genet Epidemiol* 2000; 19: Suppl. 1, S36–S42.

35. Lange C, Laird NM. Power calculations for a general class of family-based association tests: dichotomous traits. *Am J Hum Genet* 2002; 71: 575–584.

36. 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; 13: 723–726.

37. Garrib A, Zhou W, Sherwood R, Peters TJ. Angiotensin-converting enzyme (ACE) gene polymorphism in patients with sarcoidosis. *Biochem Soc Trans* 1998; 26: S137.

38. Papadopoulos KI, Melander O, Orho-Melander M, Groop LC, Carlsson M, Hallengren B. Angiotensin converting enzyme (ACE) gene polymorphism in sarcoidosis in relation to associated autoimmune diseases. *J Intern Med* 2000; 247: 71–77.

39. Sharma P, Smith I, Maguire G, Stewart S, Shneerson J, Brown MJ. Clinical value of ACE genotyping in diagnosis of sarcoidosis. *Lancet* 1997; 349: 1602–1603.

40. Takemoto Y, Sakatani M, Takami S, *et al.* Association between angiotensin II receptor gene polymorphism and serum angiotensin converting enzyme (SACE) activity in patients with sarcoidosis. *Thorax* 1998; 53: 459–462.

41. Guleva I, Seitzer U. Vitamin D receptor gene polymorphism in patients with sarcoidosis. *Am J Respir Crit Care Med* 2000; 162: 760–761.

42. Swider C, Schnittger L, Bogunia-Kubik K, *et al.* TNF-alpha and HLA-DR genotyping as potential prognostic markers in pulmonary sarcoidosis. *Eur Cytokine Netw* 1999; 10: 143–146.

43. Edmondstone WM, Wilson AG. Sarcoidosis in Caucasians, Blacks and Asians in London. *Br J Dis Chest* 1985; 79: 27–36.

44. Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AV. Association of vitamin D receptor genotype with leprosy type. *J Infect Dis* 1999; 179: 187–191.

45. Bellamy R, Ruwende C, Corrah T, *et al.* Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* 1999; 179: 721–724.

46. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. Association mapping in structured populations. *Am J Hum Genet* 2000; 67: 170–181.