

## Role of genetic polymorphisms in ACE and TNF- $\alpha$ gene in sarcoidosis: a meta-analysis

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**Abstract** A great number of association studies have been performed to identify the genes involved in the etiology and prognosis of sarcoidosis. We performed a systematic review of case-control studies through the PubMed database and evaluated them for a possible inclusion into a meta-analysis in order to assess whether the reported genetic polymorphisms are the risk factors of sarcoidosis. Case-control studies with clear diagnostic criteria and interventions were included. Only investigations of a single polymorphism/gene involvement in sarcoidosis reported more than five times were selected. Aggregating data from 12 studies on ID/ACE polymorphisms, the odds ratio (OR) for sarcoidosis, if the polymorphism was considered under the dominant genetic model, was not significantly increased: 1.19 (95% CI 0.98–1.43); OR under the recessive model was 1.20 (95% CI 0.98–1.46). In seven case-control studies on  $-308$ /TNF- $\alpha$  polymorphism, the OR for sarcoidosis if the polymorphism considered under the dominant genetic model was significantly increased at 1.47 (95% CI 1.03–2.08); the OR under the recessive model was 1.39 (95% CI 0.67–2.90). In conclusion, the results showed that the TNF- $\alpha$  genotype could be a significant risk factor for sarcoidosis, whereas the risk of sarcoidosis due to the ACE genotype was not substantially elevated.

**Keywords** Sarcoidosis · Genetic polymorphism · ACE gene · TNF- $\alpha$  gene · Meta-analysis

### Introduction

Sarcoidosis is a chronic, multisystem disease characterised by the presence of non-caseating granulomas. It has a worldwide distribution with regional variations in frequency, affecting from less than 1 to 64 per 100,000. Adults under the age of 40 are most commonly affected. Sarcoidosis has a non-typical unpredictable clinical course, ranging from complete spontaneous remission to chronic progressive course resulting in organ failure. The lungs and lymph nodes are most commonly affected, followed in frequency by skin, eye, cardiac and neurologic involvement. Despite treatment with corticosteroids, some patients follow a chronic progressive course, and the overall mortality is estimated to be 1–5% (Hunninghake et al. 1999).

Although the etiology has not yet been elucidated, the pathophysiology of the disorder can be described as an antigen-driven process. The granulomatous lesions are the consequence of an exaggerated immunological response in genetically susceptible individuals exposed to persisting, so far not definitively defined, environmental antigens: infectious agents, such as *Mycobacterium tuberculosis*, nontuberculous mycobacteria, *Propionibacterium acnes*, other bacteria and several viruses (McGrath et al. 2001; Hance 1998) or non-infectious agents, such as beryllium, other metals, clay and pollen (Hunninghake et al. 1999).

In sarcoidosis, the inflammatory response is characterized by proliferation and activation of T<sub>H</sub>1 cells and increased production of proinflammatory cytokines, as well as fibrogenic mediators that amplify the immune response, resulting finally in granulomatous inflammation (Hunninghake et al. 1999; McGrath et al. 2001; Hance 1998; Muller-Quernheim 1998). Among various cytokines influencing formation and maintenance of granuloma, it has been proposed that interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-2 (IL-2), interleukin-12 (IL-12),

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interleukin-15 (IL-15) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) play a central role in this process. Besides, other mediators are involved in granuloma formation and persistence such as angiotensin-converting enzyme (ACE) (Luisetti et al. 2000; Baughman et al. 2003).

Genetic susceptibility to sarcoidosis has been demonstrated through differences in the prevalence among different populations and ethnic groups, and through familial aggregation of the disease, its similarity to other hereditary granulomatous disorders, through association with HLA genes, and significant linkage of the disease to several loci, most notably to the HLA region on the short arm of the chromosome 6 (Schurmann et al. 2000; Rybicki et al. 2001; Rybicki et al. 1997). The pattern of inheritance in familial cases suggests a multifactorial model, whereby genetic predisposition at multiple genetic loci interacts with environmental factors resulting in sarcoidosis. A number of association studies were performed with the aim of elucidating this genetic background, but the results were inconclusive.

In order to identify a possible genetic risk of sarcoidosis, and to avoid limitations arising from individual studies related to small samples and false positive or negative findings, we performed a comprehensive meta-analysis of case-control studies of genetic polymorphisms involved in sarcoidosis.

## Materials and methods

### Search strategy

The electronic database PubMed MEDLINE (www.pubmed.gov) was searched up until June 2006 for studies on candidate genes in sarcoidosis. Our investigation was based on the medical subject heading terms: *sarcoidosis and polymorphism*, *sarcoidosis and genetics*, *sarcoidosis and gene*. All genetic association studies evaluating any candidate gene in sarcoidosis were registered, and also the review articles. Initially, all languages were considered. Special consideration was dedicated to case-control studies. To avoid a possible loss of any relevant article, an additional control was performed through the references cited in identified articles, through the link “related articles” offered in the PubMed database, and through the references of review articles. Finally, a new additional article search was performed in PubMed with new medical subject heading terms: sarcoidosis and the name of the individual candidate gene detected on primary investigation.

### Study selection

After the search through the key words sarcoidosis and polymorphism, 155 studies were identified. Only studies

analyzing the association between sarcoidosis and gene polymorphisms were analyzed further: 53 studies analyzing 22 gene polymorphisms were detected, as shown in Table 1.

Further selection of these studies was based on the following inclusion criteria: retrospective case-control studies with clear diagnostic criteria of sarcoidosis and genotype frequencies reported. The studies evaluating patients with a distinct clinical form of sarcoidosis were also included. The studies reporting allele frequencies without genotype frequencies were excluded. Abstracts and letters, editorials and reviews, and congress communications were excluded. The studies performed by the same group of authors were controlled for a possible overlapping patient inclusion. Doing this we found that no meta-analysis on sarcoidosis and polymorphisms has been published so far. Finally, only polymorphisms in genes investigated in at least five studies were included in the meta-analysis. The studies were analyzed by two authors independently (I.M. and A.K.). On the basis of these criteria, only the studies dealing with a single polymorphism in the ACE gene and a single polymorphism in the TNF- $\alpha$  gene were selected for the final evaluation.

### Data extraction

The final review was performed on 17 studies analyzing polymorphisms in the ACE gene, 12 of them being case control studies on insertion/deletion (ID) polymorphisms in intron 16, and these were submitted for meta-analysis (Arbustini et al. 1996; Furuya et al. 1996; Sharma et al. 1997; Tomita et al. 1997; Takemoto et al. 1998; Garrib et al. 1998; Maliarik et al. 1998; Pietinalho et al. 1999; Papadopoulos et al. 2000; McGrath et al. 2001; Planck et al. 2002; Alia et al. 2005). The remaining five studies not included were family-based studies, studies evaluating serum ACE levels or studies evaluating sarcoidosis symptoms (Csaszar et al. 1997; Niimi et al. 1998; Kawakami et al. 1998; Schurmann et al. 2001; Rybicki et al. 2004).

Of nine studies on TNF- $\alpha$  polymorphisms in sarcoidosis, seven case-control studies on -308 polymorphism were included in the meta-analysis (Seitzer et al. 1997; Takashige et al. 1999; Somskövi et al. 1999; Yamaguchi et al. 2001; Labunski et al. 2001; Pandey et al. 2002; Mrazek et al. 2005). One excluded study reported allelic frequencies; the other was family-based study (Rybicki et al. 2004; Grutters et al. 2002).

Additionally, there were five case-control studies identified that reported an association of intron 1/tumor necrosis factor- $\beta$  (TNF- $\beta$ ) polymorphism and sarcoidosis (Seitzer et al. 1997; Takashige et al. 1999; Yamaguchi et al. 2001; Mrazek et al. 2005; Ishihara et al. 1995), but only three included the data on genotype frequencies.

**Table 1** Candidate genes and their polymorphisms investigated in sarcoidosis

| Gene              | Protein function    | Polymorphism                    | No. of studies (References)   | Case-control studies  |
|-------------------|---------------------|---------------------------------|---|---|
| ACE               | Proteinase          | I/D                             | 17 (Arbustini et al. 1996; Furuya et al. 1996; Sharma et al. 1997; Tomita et al. 1997; Takemoto et al. 1998; Garrib et al. 1998; Maliarik et al. 1998; Pietinalho et al. 1999; Papadopoulos et al. 2000; McGrath et al. 2001; Planck et al. 2002; Alia et al. 2005; Csaszar et al. 1997; Niimi et al. 1998; Kawakami et al. 1998; Schurmann et al. 2001; Rybicki et al. 2004) | 12 (Arbustini et al. 1996; Furuya et al. 1996; Sharma et al. 1997; Tomita et al. 1997; Takemoto et al. 1998; Garrib et al. 1998; Maliarik et al. 1998; Pietinalho et al. 1999; Papadopoulos et al. 2000; McGrath et al. 2001; Planck et al. 2002; Alia et al. 2005) |
|                   |                     | A-5466C                         | 1 (Schurmann et al. 2001)   |   |
|                   |                     | 4656(CT)2/3                     | 1 (Schurmann et al. 2001)   |   |
| TNF $\alpha$      | Cytokine            | -308                            | 9 (Rybicki et al. 2004; Seitzer et al. 1997; Takashige et al. 1999; Somskövi et al. 1999; Yamaguchi et al. 2001; Labunski et al. 2001; Grutters et al. 2002; Pandey et al. 2002; Mrazek et al. 2005)  | 7 (Seitzer et al. 1997; Takashige et al. 1999; Somskövi et al. 1999; Yamaguchi et al. 2001; Labunski et al. 2001; Pandey et al. 2002; Mrazek et al. 2005)   |
|                   |                     | -238                            | 3 (Yamaguchi et al. 2001; Grutters et al. 2002; Pandey et al. 2002)   |   |
|                   |                     | -1031                           | 1 (Grutters et al. 2002)  |   |
|                   |                     | -863                            | 1 (Grutters et al. 2002)  |   |
|                   |                     | -857                            | 1 (Grutters et al. 2002)  |   |
|                   |                     | -244                            | 1 (Yamaguchi et al. 2001)   |   |
| TNF $\beta$ (LTA) | Cytokine            | Intron1 NCOI                    | 5 (Seitzer et al. 1997; Takashige et al. 1999; Yamaguchi et al. 2001; Mrazek et al. 2005; Ishihara et al. 1995)   | 5 (Seitzer et al. 1997; Takashige et al. 1999; Yamaguchi et al. 2001; Mrazek et al. 2005; Ishihara et al. 1995)   |
| IL-1 $\beta$      | Cytokine            | Exon5/TaqI +3953                | 3 (Pandey et al. 2002; Niimi et al. 2000; Hutyorova et al. 2002)  |   |
|                   |                     | -511                            | 2 (Pandey et al. 2002; Hutyorova et al. 2002)   |   |
| IL-18             | Cytokine            | -607                            | 3 (Takada et al. 2002; Janssen et al. 2004; Zhou et al. 2005)   |   |
|                   |                     | -137                            | 3 (Takada et al. 2002; Janssen et al. 2004; Zhou et al. 2005)   |   |
|                   |                     | -656                            | 2 (Janssen et al. 2004; Zhou et al. 2005)   |   |
|                   |                     | 1248                            | 1 (Janssen et al. 2004)   |   |
| BTNL2             | Ig gene superfamily | Np16071 (A/G)/ rs 2076350/exon5 | 3 (Valentonyte et al. 2005; Rybicki et al. 2005; Li et al. 2006)  |   |
| CARD15 (NOD2)     |                     | 19 SNPs                         | 3 (Martin et al. 2003; Schurmann et al. 2003; Kanazawa et al. 2005)   |   |
| TGF $\beta$ 1     | Cytokine            | Codon 25 (Arg25Pro)             | 1 (Muraközy et al. 2001)  |   |
|                   |                     | Codon 10 T869C (Leu10Pro)       | 1 (Niimi et al. 2002)   |   |
|                   |                     | 5 SNPs                          | 1 (Kruit et al. 2006)   |   |
| CCR2              | Chemokine receptor  | G190A/ V64I                     | 1 (Hizawa et al. 1999; Petrek et al. 2000; Spagnolo et al. 2003; Valentonyte et al. 2005)   |   |

**Table 1** continued

| Gene | Protein function     | Polymorphism | No. of studies (References)                      | Case-control studies |
|------|----------------------|--------------|--|----------------------|
|      |                      | 8 SNPs       | 1 (Spagnolo et al.2003; Valentonyte et al. 2005) |                      |
| TAP1 | MHC gene superfamily | Ile333Asp    | 3 (Ishihara et al.1996; Foley et al. 1997,1999)  |                      |
|      |                      | Asp637Gly    | 3 (Ishihara et al.1996; Foley et al.1997,1999)   |                      |

CCR5, IL-1Ra, IL-1 $\alpha$ , CTLA4, CC10, LMP7, IFN $\gamma$ , HSP70, Ig (GM, KM), VDR, AGTIIR1, AGTIIR2

ACE angiotensin I converting enzyme, *TNF $\alpha$*  tumor necrosis factor alpha, *TNF $\beta$*  tumor necrosis factor beta, *LTA*lymphotoxin alpha, *IL-1 $\beta$*  interleukin 1 beta, *IL-18* interleukin 18, *BTNL2* butyrophilin-like 2, *CARD15* caspase recruitment domain family, member 15, *NOD2* nucleotide-binding oligomerization domain containing 2, *TGF $\beta$ 1* transforming growth factor, beta 1, *CCR2* chemokine receptor 2, *TAP1* transporter associated with antigen processing 1, *CCR5* chemokine receptor 5, *IL-1Ra* interleukin 1 receptor alpha, *IL-1 $\alpha$*  interleukin 1 alpha, *CTLA4* cytotoxic T-lymphocyte-associated protein 4, *CC10*clara-cell specific 10-kD protein, *LMP7* low molecular weight protein 7, *IFN $\gamma$*  interferon gamma, *HSP70* heat shock 70kDa protein, *Ig* (GM, KM) immunoglobulin G heavy chain, K light chain, *VDR* vitamin D receptor.*AGTIIR1* angiotensin II receptor, type 1, *AGTIIR2* angiotensin II receptor, type 2

All remaining studies on other gene polymorphisms were not further evaluated as they dealt with polymorphisms rarely investigated, each of them reported less than five times.

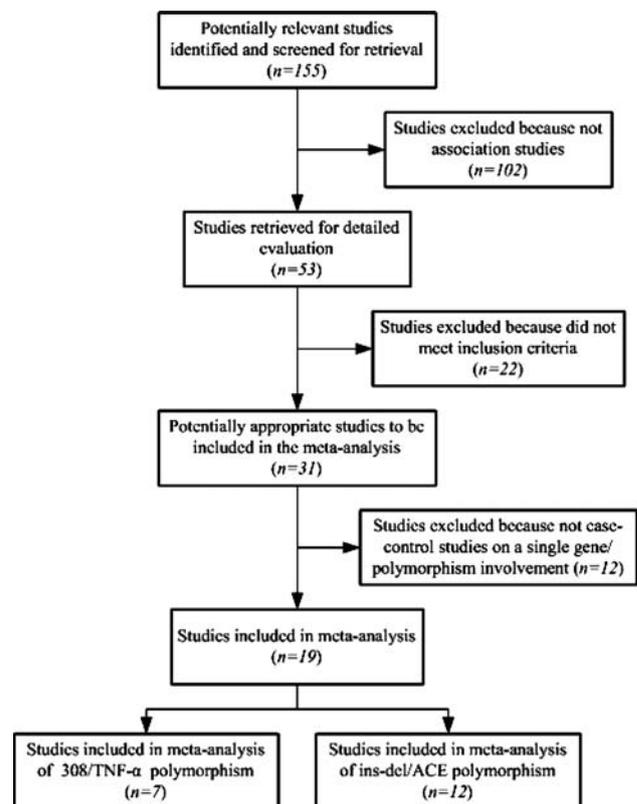
The meta-analysis was performed for studies on ID/ACE polymorphism and for studies on -308/TNF- $\alpha$  polymorphism. For each polymorphism/sarcoidosis analysis the following data were extracted: author, country and year of publication, patients' and controls' ethnicity and continent, clinical form, patients' and controls' mean age if reported, and clinical diagnostic criteria. For each polymorphism/sarcoidosis analysis, the odds ratio (OR) was calculated under both assumptions: the supposed effect of polymorphism if dominant, and its effect if recessive.

**Data analysis**

Fisher's exact test was applied to testing for Hardy-Weinberg proportions. For each study on genetic variants, individual and pooled odds ratio and associated 95% confidence intervals were calculated, using the fixed-effects model (Mantel-Haenszel method) and random-effects model (DerSimonian-Laird method). P values less than 0.05 were set as significant. The Cochrane Q test for heterogeneity with a 10% significance level was used to assess whether variability among studies was greater than expected by chance alone. In addition, I<sup>2</sup> was used to describe the percentage of variation across studies due to heterogeneity rather than chance. For assessment of publication bias the funnel plot was used. All statistical computing and graphics were carried out in R, version 2.4.1 (www.r-project.org).

**Results**

The genetic associations between sarcoidosis and polymorphism genotypes of ACE and TNF- $\alpha$  gene were examined. More than five association studies were found for the intron 16 ID/ACE gene polymorphism (12 studies) and for the TNF- $\alpha$ /-308 G→A polymorphism (7 studies) (Fig. 1).



**Fig. 1** Flow diagram of study selection for the meta-analyses

All these 19 studies had clearly defined diagnostic criteria and genotype frequencies. For both polymorphisms the statistical analysis was performed under both the dominant and recessive genetic model. The genotype distribution among control subjects in each study did not deviate from the expected Hardy-Weinberg equilibrium.

### 1. Results of ID/ACE polymorphism in sarcoidosis

The characteristics of 12 analyses (Arbustini et al. 1996; Furuya et al. 1996; Sharma et al. 1997; Tomita et al. 1997; Takemoto et al. 1998; Garrib et al. 1998; Maliarik et al. 1998; Pietinalho et al. 1999; Papadopoulos et al. 2000; McGrath et al. 2001; Planck et al. 2002; Alia et al. 2005) on the risk of sarcoidosis in subjects with intron16 ID polymorphism in the ACE gene are summarized in Table 2. These studies involved 1,392 patients and 2,147 controls. Two studies reported two different ethnic groups; therefore, 14 ethnic groups were analyzed. All but two studies reported genotype frequencies in absolute numbers; in two studies genotype frequencies were extrapolated from genotype percentages.

The summary OR under a fixed-effects assumption when comparing homozygous carriers of the mutation plus heterozygous carriers (DD + ID) versus homozygous carriers of the wild type allele (II) was 1.20 (95% CI 1.02–1.41). The estimate based on the random-effect assumption was 1.19 (95% CI .98–1.43). Pooled data indicated no heterogeneity:  $\chi^2(13) = 16$  ( $p = 0.249$ );  $I^2 = 18.8\%$ . The distribution of the OR in relation to its standard error in the funnel plot was symmetrical, suggesting a low probability of publication bias.

When comparing homozygous mutant type allele carriers (DD) versus heterozygous plus homozygous wild type (ID + II) carriers (recessive model), the summary OR under a fixed-effect model was 1.19 (95% CI 1.01–1.41); the estimates under a random-effect model was 1.20 (95% CI .98–1.46). No heterogeneity was observed:  $\chi^2(13) = 17.08$  ( $P = 0.196$ );  $I^2 = 23.9\%$ . The distribution of the OR in relation to its standard error in the funnel plot was symmetrical, suggesting a low probability of publication bias.

Figure 2 shows the results of individual and summary OR estimates with 95% CI (the fixed-effect model): (a) dominant genetic model; (b) recessive genetic model.

### 2. Results of -308/TNF- $\alpha$ polymorphism in sarcoidosis

The characteristics of seven analyses (Seitzer et al. 1997; Takashige et al. 1999; Somskövi et al. 1999; Yamaguchi et al. 2001; Labunski et al. 2001; Pandey et al. 2002; Mrazek et al. 2005) on the risk of sarcoidosis in subjects

with the -308 polymorphism in the TNF- $\alpha$  gene are summarized in Table 3. These studies involved 901 patients and 1,459 controls. One study reported two different ethnic groups; therefore, eight ethnic groups were analyzed.

The summary OR under a fixed-effect assumption when comparing homozygous carriers of the mutation plus heterozygous carriers (2/2 + 1/2) versus homozygous carriers of the wild type allele (1/1) was 1.33 (95% CI 1.09–1.62). The estimate based on the random-effect assumption was 1.47 (95% CI 1.03–2.08). The test for heterogeneity was significant:  $\chi^2(7) = 15.97$  ( $P = 0.025$ );  $I^2 = 56.2\%$ . The distribution of the OR in relation to its standard error in the funnel plot was symmetrical, suggesting a low probability of publication bias.

When comparing homozygous mutant type allele carriers (2/2) versus heterozygous plus homozygous wild type (1/2 + 1/1) carriers (recessive model), the summary OR under a fixed-effect model was 1.47 (95% CI 0.91–2.37); the estimates under a random-effect model was 1.39 (95% CI 0.67–2.90). The test for heterogeneity was not significant:  $\chi^2(7) = 10.56$  ( $P = 0.159$ );  $I^2 = 33.7\%$ . The distribution of the OR in relation to its standard error in the funnel plot was symmetrical, suggesting a low probability of publication bias.

Figure 3 shows the results of individual and summary OR estimates with 95% CI (the fixed-effect model): (a) dominant genetic model; (b) recessive genetic model.

## Discussion

In our systematic review of studies on involvement of genetic polymorphisms in sarcoidosis etiology, we made a meta-analysis only on two widely investigated genes: ACE and TNF- $\alpha$ , and their respective single polymorphisms, as they were systematically investigated in more than five studies. Both genes are involved in immunological overreaction and granuloma formation typical for sarcoidosis. The results of our meta-analysis do not demonstrate a significant association of the ID polymorphism in the ACE gene either for the recessive or for the dominant genetic model. A small (47%), but statistically significant association was demonstrated for the 308/TNF- $\alpha$  gene polymorphism under the dominant genetic model.

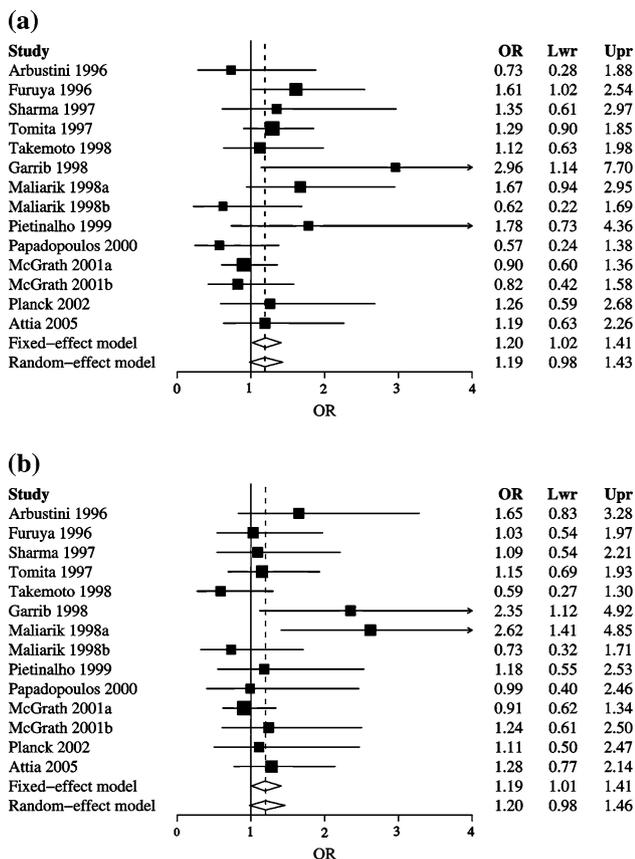
For the meta-analysis of ID/ACE polymorphism involvement in sarcoidosis, 12 studies on 14 populations were available. The summary OR demonstrates that homozygous carriers of the deletion allele have a slight (20%), but statistically not significantly increased risk of sarcoidosis; the same result was obtained when the homozygous plus heterozygous carriers of the deletion allele were combined. In individual studies performed in

**Table 2** Characteristics of studies on the association between the ACE insertion/deletion (ID) polymorphism and sarcoidosis

| Study, country, ethnicity     | Cases: number and genotypes               | Controls: number and genotypes            | Comments   |
|-------------------------------|---|---|--|
| Arbustini et al. 1996         | 61, mean age 35.3 years                   | 80, mean age 35 years                     | Patients ascertainment/diagnostic criteria proper<br>HW+<br><i>P</i><br>Also: genotype vs. sACE levels                         |
| Italy                         | DD 27                                     | DD 26                                     |  |
| Italian                       | ID 24                                     | ID 44                                     |  |
| Europe                        | II 10                                     | II 10                                     |  |
| Furuya et al. 1996            | 103, mean age 39.5 years                  | 341, mean age 42.5 years                  | Patients ascertainment/diagnostic criteria proper<br>OR<br>Also: genotype vs. sACE levels                                      |
| Japan                         | DD 14                                     | DD 45                                     |  |
| Japanese                      | ID 53                                     | ID 138                                    |  |
| Asia                          | II 36                                     | II 158                                    |  |
| Sharma et al. 1997            | 47, mean age 47 years                     | 146, mean age 41.4 years                  | Patients ascertainment/diagnostic criteria proper<br>Research letter<br>Not a case-control study, but data reported adequately |
| UK                            | DD 15                                     | DD 44                                     |  |
| White                         | ID 22                                     | ID 63                                     |  |
| Europe                        | II 10                                     | II 39                                     |  |
| Tomita et al. 1997            | 207, mean age 56.2 years                  | 314, mean age 51.3 years                  | Patients ascertainment/diagnostic criteria proper<br>HW+<br><i>P</i><br>Also: genotype vs. sACE levels; genot. vs. clin course |
| Japan                         | DD 29                                     | DD 39                                     |  |
| Japanese                      | ID 101                                    | ID 139                                    |  |
| Asia                          | II 77                                     | II 136                                    |  |
| Takemoto et al. 1998          | 100, mean age 49.4 years                  | 96, mean age 51.6 years                   | Patients ascertainment/diagnostic criteria proper<br><i>P</i><br>Also: genotype vs. sACE levels                                |
| Japan                         | DD 12                                     | DD 18                                     |  |
| Japanese                      | ID 50                                     | ID 39                                     |  |
| Asia                          | II 38                                     | II 39                                     |  |
| Garrib et al. 1998            | 54, mean age ?                            | 100, mean age 38.2 years                  | Patients ascertainment/ diagnostic criteria proper<br>HW+<br><i>P</i><br>Numbers in percentages!                               |
| UK                            | DD 20                                     | DD 20                                     |  |
| Caucasians                    | ID 28                                     | ID 53                                     |  |
| Europe                        | II 6                                      | II 27                                     |  |
| Maliarik et al. 1998          | 183 African American, mean age 47.2 years | 111 African American, mean age 39.1 years | Patients ascertainment/diagnostic criteria proper<br>OR<br>Also: genot. vs. clin course<br>Numbers in percentages!             |
| USA                           | DD 56                                     | DD 16                                     |  |
| African Americans, Caucasians | ID 95                                     | ID 66                                     |  |
| America                       | II 32                                     | II 29                                     |  |
|                               | 60 Caucasian, mean age 47.1 years         | 48 Caucasian, mean age 39.1 years         |  |
|                               | DD 15                                     | DD 15                                     |  |
|                               | ID 32                                     | ID 26                                     |  |
|                               | II 13                                     | II 7                                      |  |
| Pietinalho et al. 1999        | 59  | 70  | Patients ascertainment/diagnostic criteria proper<br>OR, <i>P</i><br>Also: genot. vs. clin course                              |
| Finland, Japan                | DD 18                                     | DD 19                                     |  |
| Finnish                       | ID 32                                     | ID 34                                     |  |
| Europe                        | II 9                                      | II 17                                     |  |
| Papadopoulos et al. 2000      | 32, mean age 44.5 years                   | 107, mean age 58 years                    | Patients ascertainment/diagnostic criteria proper<br><i>P</i>  |
| Sweden                        | DD 8                                      | DD 27                                     |  |
| Caucasian (-1)                | ID 14                                     | ID 58                                     |  |
| Europe                        | II 10                                     | II 22                                     |  |

**Table 2** continued

| Study, country, ethnicity | Cases: number and genotypes    | Controls: number and genotypes | Comments   |
|---------------------------|--------------------------------|--------------------------------|--|
| McGrath et al. 2001       | 180 British, mean age 40 years | 386 British                    | Patients ascertainment/diagnostic criteria proper  |
| UK, Czech Rep             | DD 51                          | DD 117                         |  |
| Caucasian white           | ID 84                          | ID 180                         | <i>P</i>   |
| Europe                    | II 45                          | II 89                          | Also: genot. vs. clin course                       |
|                           | 56 Czech, mean age 46 years    | 179 Czech                      |  |
|                           | DD 14                          | DD 38                          |  |
|                           | ID 25                          | ID 94                          |  |
|                           | II 17                          | II 47                          |  |
| Planck et al. 2002        | 73, mean age 38 years          | 65, mean age 32 years          | Patients ascertainment/diagnostic criteria proper  |
| Sweedden, Japan           | DD 17                          | DD 14                          |  |
| Scandinavian              | ID 38                          | ID 32                          | <i>P</i>   |
| Europe                    | II 18                          | II 19                          | Also: genot. vs. clin course                       |
|                           |                                |                                | Numbers in percentages!                            |
| Alia et al. 2005          | 177, mean age 51 years         | 104, mean age 44 years         | Patients ascertainment/ diagnostic criteria proper |
| Spain                     | DD 68                          | DD 34                          |  |
| Spanish                   | ID 81                          | ID 51                          | <i>P</i> , OR                                      |
| Europe                    | II 28                          | II 19                          | Also: genot. vs. clin course                       |



**Fig. 2** The results of individual and summary OR estimates with 95% CI, fixed-effect model: **(a)** ID/ACE polymorphism—dominant genetic model; **(b)** ID/ACE polymorphism—recessive genetic model. The size of the square is proportional to the percent weight of each study; horizontal lines represent the 95% CI

Caucasians (Italian, UK twice, American Caucasians, Finnish, Swedish twice, Czechs, and Spanish) and in Japanese (three times), the association between the polymorphism and sarcoidosis was not demonstrated. There have been speculations that the polymorphism should be involved in the pathogenesis of sarcoidosis in Japanese female patients (Furuya et al. 1996), in poor prognosis in Finnish patients (Pietinalho et al. 1999) and in familial sarcoidosis (Maliarik et al. 1998; Schurmann et al. 2001). However, only in a study on African Americans (Maliarik et al. 1998) was the association found, this result being in favor of specific ethnicity/risk association of sarcoidosis.

ACE is a zinc-metalloproteinase present in the epithelial cells of proximal convoluted kidney tubules and in the luminal surface of lung endothelial cells (Ehleres and Riordan 1989). Since the evidence has been brought that ACE is produced by epitheloid cells of sarcoid granulomas, and after the elevated serum ACE levels (sACE) were found in patients with sarcoidosis, sACE has been proposed to be the marker of sarcoidosis activity, susceptibility and prognosis (Pertschuk et al. 1981; Lieberman 1975; Meeting Report 1994). It has also been demonstrated that ACE synthesis in a monocyte cell culture can be modulate by T lymphocytes obtained from sarcoid patients (Silverstein et al. 1979). It is now believed that sACE reflects the whole body granuloma mass (Ainslie et Benatar 1985). The level of serum and intracellular ACE is influenced by a 287-bp ID polymorphism in intron 16 of the ACE gene: possession of D allele is associated with

**Table 3** Characteristics of studies on the association between the TNF- $\alpha$ -308 G (1)  $\rightarrow$  A (2) polymorphism and sarcoidosis

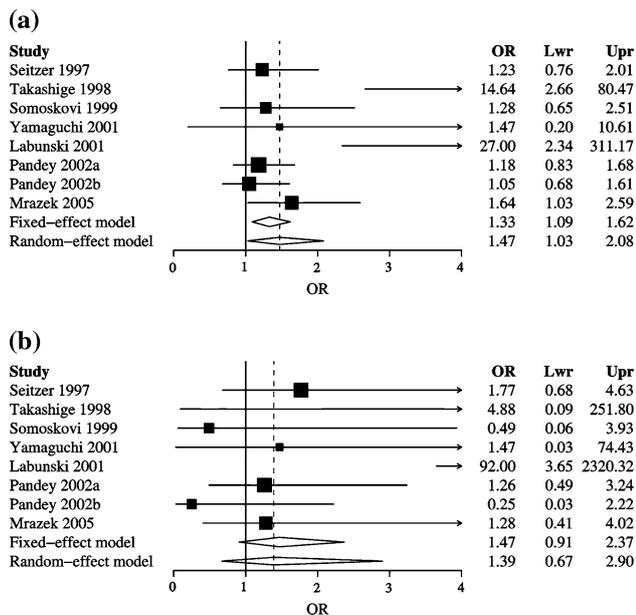
| Study, country, ethnicity    | Cases: number and genotypes | Controls: number and genotypes | Comments  |
|------------------------------|-----------------------------|--------------------------------|---|
| Seitzer et al. 1997          | 101                         | 216                            | Patients ascertainment/diagnostic criteria proper |
| Germany                      | 11 62                       | 11 143                         |   |
| Germans                      | 12 31                       | 12 63                          | <i>P</i>  |
| Europe                       | 22 8                        | 22 10                          | Also: genot. vs. clin course                      |
| Takashige et al. 1998        | 26                          | 125                            | Patients ascertainment/diagnostic criteria proper |
| Japan                        | 11 21                       | 11 123                         |   |
| Japanese                     | 12 5                        | 12 2                           | Cardiac sarc.patients                             |
| Asia                         | 22 0                        | 22 0                           |   |
| Somoskövi et al. 1999        | 43                          | 216                            | Patients ascertainment/diagnostic criteria proper |
| Germany                      | 11 26                       | 11 143                         |   |
| Germans                      | 12 16                       | 12 63                          |   |
| Europe                       | 22 1                        | 22 10                          |   |
| Yamaguchi et al. 2001        | 110, mean age 39.4 years    | 161                            | Patients ascertainment/diagnostic criteria proper |
| Japan                        | 11 108                      | 11 159                         |   |
| Japanese                     | 12 2                        | 12 2                           | <i>P</i> , OR                                     |
| Asia                         | 22 0                        | 22 0                           | Also: genot. vs. clin course                      |
| Labunski et al. 2001         | 10                          | 12 patients- drug eruption     | Patients ascertainment/diagnostic criteria proper |
| Germany                      | 11 1                        | 11 9                           |   |
| German                       | 12 1                        | 12 3                           | Lofgren sarc. patients                            |
| Europe                       | 22 8                        | 22 0                           |   |
| Pandey et al. 2002           | 278 Caucasians              | 278                            | Patients ascertainment/diagnostic criteria proper |
| USA                          | 11 182                      | 11 192                         |   |
| Caucasian, African Americans | 12 86                       | 12 78                          | HW+   |
| America                      | 2 2 10                      | 22 8                           | <i>P</i>  |
|                              | 219 African Americans       | 219                            |   |
|                              | 11 161                      | 11 163                         |   |
|                              | 12 57                       | 12 52                          |   |
|                              | 22 1                        | 22 4                           |   |
| Mrazek et al. 2005           | 114, mean age 44.8 years    | 232, mean age 33.8 years       | Patients ascertainment/diagnostic criteria proper |
| Czech Republic, UK           | 11 64                       | 11 157                         |   |
| Czechs                       | 12 45                       | 12 67                          | <i>P</i>  |
| Europe                       | 22 5                        | 22 8                           | Also: genot. vs. clin course                      |

higher production of sACE in both the general population and sarcoidosis patients (Rigat et al. 1990; Tirt et al. 1992; Arbustini et al. 1996; Furuya et al. 1996; Sharma et al. 1997; Tomita et al. 1997; Csaszar et al. 1997). All these findings have contributed to the idea of the ID/ACE polymorphism's involvement in sarcoidosis etiology and/or pathogenesis, but neither the individual studies (except for African Americans) nor our meta-analysis confirmed this idea.

However, ACE genotyping is necessary for precise assessment of sACE levels, and it increases its role in diagnostic evaluation of sarcoidosis patients. Namely, the studies of sACE in sarcoidosis demonstrated an increased level in about 60% of patients. Such a poor sensitivity increases if taking into consideration the underlying ID

genotype: genotype-specific levels of sACE are much more sensitive (Arbustini et al. 1996; Furuya et al. 1996; Sharma et al. 1997; Tomita et al. 1997; Sharma and Said 1995; Costabel and Teschler 1997).

For the meta-analysis of -308/TNF- $\alpha$  polymorphism involvement in sarcoidosis, seven studies on eight populations were available. The summary OR demonstrated that combined homozygous plus heterozygous carriers of the mutant allele (dominant genetic model) have 47% increased risk of sarcoidosis. The interpretation of the recessive genetic model is difficult due to a small number of mutant homozygotes in most studies, both in patients and in controls. In individual case-control studies performed in Caucasians (Germans three times, American Caucasians, Czechs), in Japanese (twice) and in African



**Fig. 3** The results of individual and summary OR estimates with 95% CI, fixed-effect model: **(a)**  $-308$  G/A(1/2)/TNF- $\alpha$  polymorphism—dominant genetic model; **(b)**  $-308$  G/A(1/2)/TNF- $\alpha$  polymorphism—recessive genetic model. The size of the square is proportional to the percent weight of each study; horizontal lines represent the 95% CI

Americans, the association between the polymorphism and sarcoidosis was not demonstrated, except for the study on Czech patients (Mrazek et al. 2005). However, it has been suggested that the polymorphism could be involved in clinical presentation of sarcoidosis, but these suggestions are contradictory: two studies suggested the allele 2 being associated with erythema nodosum (Löfgren syndrome), whereas one study suggested that the same allele should be associated with cardiac sarcoidosis (Seitzer et al. 1997; Takashige et al. 1999; Labunski et al. 2001). It has to be stressed that two individual reports of the  $-308$ /TNF- $\alpha$  polymorphism involvement in sarcoidosis have been performed on specific clinical presentation of sarcoidosis and on a remarkably small number of patients (Takashige et al. 1999; Labunski et al. 2001). The summary OR performed if excluding these two studies is 1.24 (95% CI 1.02–1.52).

The positive association found in this meta-analysis is in agreement with the findings of previous studies demonstrating the role of TNF- $\alpha$  gene in sarcoidosis pathogenesis: altered expression of the encoded protein combined with a demonstrated influence of the altered genotype on the pathogenesis of sarcoidosis. TNF- $\alpha$  is one of the most important cytokines in the pathogenesis of sarcoidosis, having the pivotal role in granuloma formation (Kindler et al. 1989). Increased amounts of TNF- $\alpha$  have been detected at sites of disease activity (Müller-Quernheim et al. 1992; Zheng et al. 1995). The biallelic polymorphism

at position  $-308$  of the TNF- $\alpha$  promoter, consisting of the alleles 1 (guanine at position  $-308$ ) and 2 (adenine at position  $-308$ ), has an influence on the TNF- $\alpha$  production, and allele 2 shows enhanced responsiveness after appropriate stimulation compared with more common allele 1; therefore, carriers of allele 2 might be prone to a more severe outcome of inflammatory disease and might be susceptible to autoimmune diseases due to the enhanced TNF- $\alpha$  production (Wilson et al. 1992; Bouma et al. 1996; Wilson et al. 1997; Kröger et al. 1997). The findings of our meta-analysis demonstrating an increased risk of sarcoidosis susceptibility in carriers of the allele 2 confirm this observation.

Regarding the interpretation of the meta-analyses in the light of limitations originating from original papers, efforts were made to avoid biases: study selection was rigorous, and only studies with clear diagnostic criteria reported and with reliable standardized molecular genetics methods were considered. Although some included reports dealt only with a specific clinical presentation of the disease, the diagnosis was always definitive, and thus, the participants, interventions and outcome measures among studies were similar and comparable.

Another source of variation of our analyses could be the restriction to studies published only in the English language, but, as only a few non-English mostly review articles were identified, these presumably did not influence our results. The participants of different ethnicities (Caucasians, Japanese and African Americans) were included together in the summary statistic evaluation. However, the positive association of the ACE polymorphism with sarcoidosis in the report on African Americans did not influence the final summary OR.

Regarding the statistical elaboration, all results of individual association studies were reexamined, as we performed individual OR calculation (dominant and recessive genetic model) in each study regardless of the statistical method previously applied. In both meta-analyses, funnel plots were symmetrical, and the Egger tests were not significant, indicating a low probability of publication bias. OR under the fixed-effect model and the random-effect model were calculated. No significant interstudy heterogeneity was observed for studies on ACE, recessive or dominant genetic model, and TNF- $\alpha$ , recessive genetic model. Significant study heterogeneity was observed for studies on TNF- $\alpha$ , dominant genetic model, presumably due to differences in clinical and methodological nature between studies (characteristics of the participants, study sample size). After exclusion of two studies with a small number of patients and with a specific sarcoidosis form (Takashige et al. 1999; Labunski et al. 2001), interstudy heterogeneity was no longer significant:  $\chi^2(5) = 2.1$  ( $P = 0.836$ ). A separate summary OR performed with

exclusion of these two studies was statistically significant, demonstrating a 24% increased risk of sarcoidosis in homozygous and heterozygous carriers of mutant allele.

In both meta-analyses, the aggregated number of cases was distinctively greater than the number of patients in any single study, allowing a more precise estimate of risk, which has been confirmed by our final results.

In conclusion, the results of our meta-analyses confirm that genetic causes are involved in the development of sarcoidosis. Evidence of susceptibility to sarcoidosis has been demonstrated in subject carriers of the variant in the TNF- $\alpha$  gene. The variants in the ACE gene have no effect on the risk of developing sarcoidosis. Of course, other genes, the gene–gene and gene–environment interactions also play a significant role. Additionally, our results also imply the need for further studies on the gene candidates, particularly the genes involved in immunological processes, preferably on a single functional polymorphism. However, the strategy of searching gene candidates involved in complex multifactorial diseases such as sarcoidosis should be based on integration of transcriptomic and proteomic information together with linkage analysis data and literature-based discovery knowledge, i.e., on an integrative “omic” approach.

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