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Association of angiotensin-converting enzyme/DD genotype with sarcoidosis susceptibility in Slovenian patients

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

Sarcoidosis is a multisystemic chronic inflammatory disorder of unknown etiology with multifactorial genetic predisposition. An elevated ACE serum level is considered to be the activity marker of the disease. The involvement of the ACE I/D polymorphism in sarcoidosis susceptibility has been investigated in different populations, but results have been inconclusive. The purpose of this study was to evaluate the possible association of angiotensin-converting enzyme (ACE) gene insertion (I)/deletion (D) polymorphism with sarcoidosis in the Slovene population.

Material/Methods:

In 105 sarcoidosis patients (69 female, 36 male, mean age: 41±1 years) and in 80 sex- and age-matched control subjects, genotyping for the ACE gene I/D polymorphism was performed by PCR and restriction enzyme digestion.

Results:

An increased frequency of DD homozygotes vs. II homozygotes + ID heterozygotes was found in the group of sarcoidosis patients compared with the control group (*OR*: 2.19, 95% *CI*: 1.12–4.26, *p*=0.02). No differences in genotype frequencies were found in the group of sarcoidosis patients when considering the clinical course or presentation of the disease.

Conclusions:

These results indicate that the ACE gene I/D polymorphism might be a risk factor for sarcoidosis susceptibility in the Slovene population and imply the possible role of population origin in the modulation of the influence of ACE gene variability in the pathophysiology of sarcoidosis.

key words:

angiotensin-converting enzyme (ACE) gene • insertion-deletion polymorphism • sarcoidosis

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BACKGROUND

Sarcoidosis is a chronic inflammatory disorder characterized by the presence of non-caseating epithelioid cell granulomas in multiple organs. The underlying pathophysiology of the disorder could be explained as an antigen-driven process, which in genetically susceptible persons leads to exaggerated immunological response of Th1 type [1]. The pattern of genetic susceptibility is multifactorial. A number of association studies on polymorphisms in candidate genes has been performed. Among the various clinical, immunological, biochemical, and genetic markers of sarcoidosis susceptibility, activity, clinical course, prognosis, and management, elevated serum levels of angiotensin-converting enzyme (ACE) is considered to be the activity marker which reflects whole-body granuloma load [2], another marker being tumor necrosis factor- α , which also influences the granulomatous process [3]. The level of serum ACE is influenced by a 287-bp insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene; possession of the D allele is associated with a higher production of serum ACE in both the general population and sarcoidosis patients [4–12].

The involvement of the ACE I/D polymorphism in sarcoidosis susceptibility has been investigated in several studies on different populations, but results have been inconclusive [6,7,10,13–19]. Our study was performed in order to evaluate a possible association of ACE gene I/D polymorphism and sarcoidosis susceptibility, clinical presentation, and course in a cohort of well-characterized Slovenian sarcoidosis patients.

MATERIAL AND METHODS

Patients

In the study group, 105 patients with sarcoidosis were enrolled from the out-patient clinic of the Department of Pulmonary Diseases and Allergy, UMC, Ljubljana, Slovenia. There were 69 females and 36 males, with ages ranging from 21 to 68 years and mean age at the time of diagnosis 41 ± 1 years. The patients were followed up for 9 ± 6 years after confirmation of the diagnosis. Diagnosis of sarcoidosis was based on clinical assessment, radiographic presentation, bronchoalveolar lavage, and biopsy specimens from the lung, skin, or lymph nodes after other granulomatous diseases were excluded [1]. Pulmonary lymph nodes were affected in 89 and lung parenchyma in 73 patients. According to the defined classification system [1,20], 15 patients were in stage I, 55 in stage II, 16 in stage III, and 2 in stage IV at the time of the first presentation. Extrapulmonary organ involvement was found in 35 patients. Various types of skin involvement were found in 31 patients and Löfgren syndrome was diagnosed in 19 patients. Eight patients had arthralgias. Ten patients had extrapulmonary node involvement. Five patients had salivary gland involvement. In 15 patients, involvement of parenchymal organs such as liver, spleen, kidney, or heart and in 9 patients various types of neural involvement were present. Sarcoidosis was classified as chronic if persisting more than two years and acute if it went into remission within the first two years from diagnosis [21]. Eighty healthy blood donors were the controls: 48 females and 32 males, ages ranging from 26 to 58 years and a mean age of 37 years. All patients and control subjects were Slovenian and unrelated to

each other. All participated in the study after they had given their full informed consent. The study was approved by the National Ethics Committee.

Molecular analysis

After DNA isolation from peripheral blood, polymerase chain reaction (PCR) was used to determine the ACE I/D genotype of each participant [22]. A set of primers was used to encompass the 287 base-pair (bp) insertion/deletion polymorphic region in intron 16 of the ACE gene (5'-CTGGAGACCACTCCCATCCTTCT-3' upstream primer and 5'-GATGTGGCCATCACATTCGTTCGAT-3' downstream primer). DNA was amplified for 30 cycles, with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. The PCR products were separated by electrophoresis on a 2% agarose gel and identified by ethidium bromide staining.

Statistical analysis

All data management and analyses were performed using the R language for statistical computing [23]. Differences from Hardy-Weinberg equilibrium were tested using the χ^2 test with a simulated *p*-value based on 10,000 replicates. Genotype distributions between patients and controls were compared using the χ^2 test of independence. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the risk of the disease associated with the specific genotype. The statistical power of the analysis was also calculated.

RESULTS

In the sarcoidosis patient group a higher frequency of the D allele was detected (I/D ratio 0.43:0.57), while in the control group the D allele frequency was lower (I/D ratio 0.53:0.47), the differences being not of statistical significance ($\chi^2_1=3.01$, $p=0.08$). Compared with controls, more patients were homozygous for the D allele: 37% DD homozygous among the patients vs. 21% among controls. In the sarcoidosis patient group an increased frequency of DD homozygous versus ID heterozygous plus II homozygous was found compared with the control subjects group (OR: 2.19, 95% CI: 1.12–4.26, $p=0.02$). The distributions of genotype and allele frequencies in patients and controls are shown in Table 1. The observed genotype distribution was in agreement with Hardy-Weinberg equilibrium ($\chi^2=0.22$; $p=0.66$).

Regarding polymorphism involvement in disease prognosis, no statistical difference was detected in polymorphism distribution between the 33 patients with acute course compared with the 72 patients with chronic disease. The polymorphism distribution within the sarcoidosis patients regarding clinical manifestation of the disease (19 patients with Löfgren syndrome compared with 86 non-Löfgren patients) was not of statistical significance. Distribution of genotypes, clinical course and clinical presentation is shown in Table 2.

We found no significant associations in patients between ACE gene I/D polymorphism and sex, age of onset, or organ involvement (data not shown).

The calculation of statistical power revealed that in this analysis, based on the number of 105 patients and 80 con-

Table 1. Distribution of I/D ACE genotypes and alleles in sarcoidosis patients and controls.

	Patients		Controls	
Number	105		80	
Genotypes				
			II vs. ID+DD	II+ID vs. DD
II	24 (0.23)	21 (0.26)	OR	1.20
ID	42 (0.40)	42 (0.53)	95%CI (OR)	0.61–2.36
DD	39 (0.37)	17 (0.21)	p(>OR)	0.60
			χ^2_2	0.13
			p(> χ^2_2)	0.72
				0.02
				4.71
				0.03
Alleles				
I	90 (0.43)	84 (0.53)		
D	120 (0.57)	76 (0.47)		

Table 2. Distribution of genotypes, clinical course and clinical presentation.

Number	Clinical course		Clinical presentation	
	Acute n=33	Chronic n=72	Löfgren n=19	Non-Löfgren n=86
Genotypes				
II	6	18	5	19
ID	14	28	7	35
DD	13	26	7	32
	$\chi^2_2=0.60, p=0.74$		$\chi^2_2=0.18, p=0.91$	

control subjects, the power of 80% would be achieved to detect a 2.5-fold increase in the frequencies of risk genotypes, assuming the percentage of the risk genotype in healthy control group to be 21% ($p<0.05$).

DISCUSSION

In our study of ACE I/D gene polymorphism in Slovenian sarcoidosis patients, an increased risk ($OR: 2.19, 95\% CI: 1.12-4.26, p=0.02$) of the disease susceptibility was demonstrated in homozygous carriers of the D allele (recessive genetic model). No differences in genotype frequencies were found in the group of sarcoidosis patients when considering the clinical course or clinical presentation of the disease.

ACE is involved in the pathophysiology of several pulmonary processes [24]. In sarcoidosis patients, ACE is released in excess from alveolar macrophages and epithelioid and giant cells of granulomas [25–27]. ACE is considered to be the activity marker of the disease, being the consequence of activation of the monocyte-macrophage-epithelioid cell system and reflecting the whole-body granuloma mass [2]. ACE is expressed in T lymphocytes and it further activates macrophages and augments CD4+ T-cell infiltration [28,29]. Also, angiotensin II, converted from angiotensin I by ACE, modifies several steps in inflammatory response, such as leukocyte infiltration, tissue hypertrophy, and fibrosis [30].

The D allele and the DD genotype have been reported to be a predisposing factor for cardiovascular, pulmonary, renal, immune, neurodegenerative, and metabolic diseases [24]. The I/D polymorphism and its role in the etiology of sarcoidosis have been widely investigated in association studies on various populations. Considering the high serum ACE level and its role as a biochemical marker of sarcoidosis as well as the susceptibility of serum ACE level to I/D polymorphism in the ACE gene, D variation in its homozygous form being associated with higher sACE levels, it is not surprising that the ACE gene was considered as a possible candidate gene involved in sarcoidosis etiology. Studies of the involvement of the I/D ACE gene polymorphism in sarcoidosis performed on Caucasian populations demonstrated a higher frequency of the D allele [7,14,16,18,31] among sarcoidosis patients, while studies on Japanese and Chinese populations reported a higher frequency of the I allele [6,10], which is in agreement with the already known population specificities of sarcoidosis: the disease has a higher prevalence in Caucasians than in Japanese.

The possible influence of the specific DD ACE genotype on sarcoidosis susceptibility has been not found in European and Caucasian populations such as the Italian, Finnish, Swedish, UK, Czech, and Spanish [7,14–18,31]. Also, no association was found in three studies on Japanese populations [6,10,32]. However, an association was found between the DD genotype with sarcoidosis susceptibility in Japanese female patients [6]

and in familial sarcoidosis cases in the German population [19]. On the other hand, in the study performed by Maliarik et al. on USA African-Americans, an association was found between sarcoidosis susceptibility and the homozygous DD genotype, especially when considering only familial cases [13]. Also, some studies reported an association of the polymorphism genotypes with poorer prognosis of the disease [14,15]. In our study, a statistically significantly increased frequency of DD homozygous vs. II homozygous plus ID heterozygous genotypes was demonstrated in sarcoidosis patients. The polymorphism has been widely investigated in different diseases in the Slovenian population and association has also been found for myocardial infarction/coronary heart disease and for multiple sclerosis [33,34], while no association was found for diabetic retinopathy or stroke [35,36].

The differences in findings in various reports could be the consequence of true variability among populations or population specificities such as isolation or stratification, but they could also be the consequence of imperfect molecular genetics techniques or different methodological approaches applied in different studies, such as the choice of statistical model or methodological bias due to small sample size. There is inconsistency in the statistical evaluation of ACE I/D polymorphism and its role in sarcoidosis susceptibility in the previous reports, e.g. the ORs were mostly calculated among DD or ID genotype frequencies vs. II carriers as the reference [6,13,18]. In order to avoid these contradictions, and uncertainties regarding false positive or false negative associations, and to ensure certainty in statistical analyses due to sample sizes, a meta-analysis of the already published studies on the involvement of the I/D polymorphism in the ACE gene in sarcoidosis is necessary.

The ACE I/D polymorphism has so far been investigated in numerous studies on different conditions and diseases. Although considered over-represented in genetic-association studies, its importance in physiological processes is re-emerging. The discrepancies in evaluating the biological role of ACE and those observed in population studies have been recently explained by the investigation on the role of the ACE I/D polymorphism at the cellular level, demonstrating increased angiotensin II level in ACE/DD cells and positive correlation of ACE/II cells with cell survival and cell growth. This influence of the ACE polymorphism on cell survival and on a multitude of peptides, including angiotensin II, controlling biochemical and physiological processes explains its central role in the pathophysiology of different diseases, sarcoidosis included [37,38].

CONCLUSIONS

An increased risk of sarcoidosis susceptibility was demonstrated in carriers of the DD ACE genotype in Slovenian patients. The role of the polymorphism in sarcoidosis should be further evaluated in a meta-analysis of the already published studies.

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