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ACE gene variants and sarcoidosis in a Finnish population

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Abstract. *Background:* Sarcoidosis is a systemic inflammatory disease with unknown etiology. However, there is a strong evidence of genetic influence in sarcoidosis. *Objectives:* We wanted to extend our knowledge of the role of the whole *ACE* gene, not only insertion/deletion (I/D) polymorphism, in a Finnish sarcoidosis population by genotyping the *ACE* gene region from 5' upstream to the 3' downstream. *Methods:* We genotyped 29 single nucleotide polymorphisms (SNPs) spanning the *ACE* gene from 188 sarcoidosis patients (resolved disease, n=90; persistent disease, n=98) and from 150 controls. These SNPs included tag SNP rs4343 for I/D polymorphism. To replicate the study we genotyped 11 of these SNPs from 139 Czech sarcoidosis patients (resolved disease, n=47; persistent disease, n=92) and 176 healthy controls. *Results:* No association was detected between I/D genotypes and disease susceptibility or prognosis. We found a novel SNP (rs9905945) in the 5'upstream region of the *ACE* gene to be moderately associated with favourable disease prognosis in Finnish patients [p=0.035, OR=2.034 (95%CI 1.045-3.960)]. However, in the replication study in Czechs, the SNP rs9905945 did not show association with prognosis of sarcoidosis. *Conclusions:* This study further characterizes genetic distinctions between Finnish sarcoidosis patients with different prognosis and population-specific genotype distribution of *ACE* variants. Nevertheless it seems that variants in the *ACE* gene do not considerably influence the course of the disease in Finnish sarcoidosis patients. *(Sarcoidosis Vasc Diffuse Lung Dis 2017; 34: 104-114)*

Key words: angiotensin converting enzyme (ACE), single nucleotide polymorphism (SNP), insertion/deletion (I/D) polymorphism, sarcoidosis, prognosis

Introduction

Sarcoidosis is an inflammatory disease of unknown etiology, characterized by the presence of non-caseating granulomas appearing in affected organs and resulting in multiple clinical phenotypes (1). Whatever the etiology of the disease, granuloma formation likely involves an aberrant immune response to exogenous/endogenous agents in genetically susceptible individuals (1). Clinical manifestations range from asymptomatic disease to severe loss-of-function leading to the hypothesis that sarcoidosis might not be just one disease, but consists of several distinct disease entities including an acute disease (Löfgren's syndrome), which usually resolves spontaneously, a suba-

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cute disease which also may resolve spontaneously or with treatment, and a chronic/progressive disease, each with potentially distinct genetic associations (2). This may explain why no single gene or immunological pathway defect has been found. Instead, a wide range of genes have been identified, each contributing to relatively minor effects and to a variety of clinical manifestations and differences in prognosis. Nevertheless, there is a strong evidence of genetic influence in sarcoidosis, proposed by family studies and the varying incidences in different ethnic groups (3-5).

Many genetic studies have pointed out an association between sarcoidosis and the class I, II and III genes in the major histocompatibility complex (MHC, 6p21.3) (6-11). Besides MHC genes, other genes of importance for sarcoidosis have been identified, including the *angiotensin-converting enzyme* (*ACE*) gene in chromosome 17q23. ACE converts angiotensin I into angiotensin II. Angiotensin II is a potent vasoconstrictor and the main effector molecule of the renin-angiotensin-system (RAS). Angiotensin II also has an effect in the kallikrein-kinin system which influences inflammation, hypertension, endotoxemia, and coagulopathy (7). ACE is secreted by epithelioid cells in granulomas and approximately 50% of patients with sarcoidosis have increased serum levels of ACE, making its assay probably the most widely used laboratory test for sarcoidosis (8-10). However, increased serum levels of ACE are not specific for sarcoidosis. The presence (insertion, I), or absence (deletion, D) of a 250 - 287-bp DNA fragment in intron 16 divides the *ACE* gene into two alleles (11). In relation to prognosis the insertion/deletion polymorphism in allelic (DD) or phenotypic (DD + DI) forms has been associated with sarcoidosis with varying results. However, the association between higher serum ACE levels and the allelic form (DD) has been more consistent (11-14). In a recent meta-analysis of 18 studies about I/D polymorphism the *ACE* DD genotype correlated with an increased risk of sarcoidosis (15). The controversial results of *ACE* polymorphism association to sarcoidosis may be due to mistyping of the variation. Several studies have documented problems of mistyping especially the preferential amplification of the D allele (16, 17). In a study conducted in a Caucasian population, the accuracy of the original PCR based method was only 55%, while the G allele of a single nucleotide polymorphism (SNP) rs4343 was shown to be in total linkage with the D variant yielding a genotyping accuracy of 100 % (17). In our previous study of Finnish sarcoidosis patients, the *ACE* I/D polymorphism was assessed with the traditional PCR based method. In that study the DD genotype was associated with a less favourable prognosis compared with the I/I and I/D genotypes (18).

Besides the I/D variation, little attention has so far been paid to the role of different *ACE* gene polymorphisms in relation to sarcoidosis (19). In this study we wanted to extend the knowledge of the role of the *ACE* gene in sarcoidosis. We therefore genotyped SNPs from upstream to downstream of the *ACE* gene in Finnish patients and replicated the study in another European population. The results could offer more accurate prognostic markers for evaluating the disease course.

1. Material and methods

1.1. Study subjects

Study subjects and patient characteristics have been previously described (20). In summary, we examined a total of 188 Finnish patients with verified pulmonary sarcoidosis followed-up for 5-15 years and clinically categorized into subgroups based on disease prognosis. The patients were divided into those with a disease resolved within 2 years (n=90) and to those with persisting activity after 2 years (n=98). Disease activity was determined using the generally accepted WASOG (World Association of Sarcoidosis and Other Granulomatous diseases) criteria (21). The control population consisted of 150 healthy subjects representing the Finnish population (22). ACE activities were measured from serum (S-ACE) and analyzed in different routine laboratories all around Finland. These laboratories used different methods with different reference values, thus the S-ACE levels could not be compared by absolute numbers. Instead, we grouped the values as elevated or normal according to the reference values of each laboratory. The ACE activity was measured at the point of study enrolment and patients with normal S-ACE levels at that time may have had elevated ACE activities earlier during the disease course. The S-ACE levels were assessed only in sarcoidosis sera and not in control sera.

For replication, a Czech data set (cases=139, controls=176) was used. The sarcoidosis patients had been followed for at least two years and further subdivided into those with a resolved (n=47) or a persistent disease (n=92). More detailed sample characteristics have been previously reported (23).

All study subjects were of European descent. The protocol was approved by the Ethics Committee of the Department of Internal Medicine, Hospital District of Helsinki and Uusimaa, Helsinki, Finland. In agreement with decision of this committee and Ethics Committee of University Hospital Olomouc, all probands provided written informed consent with participation in genetic association study.-

1.2. Genotyping of I/D polymorphism

We used two PCR based methods to detect the insertion/deletion polymorphism in the *ACE* gene (24, 25). Briefly, the two methods differed by primer design and amplification temperature. The consistency between SNP rs4343 genotyping (method described in 2.3.) and PCR based methods was analyzed with 10 samples for each of the genotypes (II, ID, DD) from the healthy control group. The PCR based methods for genotyping of I/D polymorphism were done only for the Finnish sample set.

1.3. Single nucleotide polymorphism genotyping

We selected 29 SNPs tagging each of the HapMap defined linkage disequilibrium (LD) blocks spanning 5-10 kb intervals from the 5' upstream to the 3' downstream region of the *ACE* gene (Table 1). These SNPs included a tag SNP rs4343 for insertion/deletion polymorphism. For replication, a subset of 11 of the 29 *ACE* SNPs were genotyped (Supplementary Table 1) from the Czech samples. This subset of SNPs was selected to cover each of the LD blocks found in Finnish samples. The SNP genotyp-

Table 1. List of single nucleotide polymorphisms (SNPs) genotyped from the Finnish samples. SNPs marked in grey did not meet genotyping criteria (minor allele frequency >0.05)

SNP	Chromosome	Position	Distance from previous SNP	Allele 1	Allele 2	Gene	Predicted function
rs9905945	chr17	61539056		C	T		
rs4968648	chr17	61541379	2323	G	A		
rs8076157	chr17	61543861	2482	T	C		
rs4459609	chr17	61548948	5087	C	A		
rs1800764	chr17	61550529	1581	C	T		
rs4291	chr17	61554194	3665	T	A	ACE	5' upstream
rs4295	chr17	61556298	2104	C	G	ACE	intronic
rs4297	chr17	61556554	256	G	C	ACE	intronic
rs3730025	chr17	61557773	1219	A	G	ACE	coding
rs4305	chr17	61558229	456	A	G	ACE	intronic
rs4309	chr17	61559923	1694	C	T	ACE	coding
rs4311	chr17	61560763	840	T	C	ACE	5' upstream
rs4321	chr17	61562774	2011	T	S	ACE	intronic
rs4341	chr17	61565990	3216	G	C	ACE	intronic
rs4343	chr17	61566031	41	G	A	ACE	coding
rs3730043	chr17	61568577	2546	C	T	ACE	coding
rs4357	chr17	61571630	3053	C	T	ACE	intronic
rs9894286	chr17	61576032	4402	C	G	ACE	3' downstream
rs4461142	chr17	61578048	2016	T	C	ACE	non-coding intronic
rs8075924	chr17	61582892	4844	C	T	ACE	non-coding intronic
rs4611524	chr17	61591652	8760	T	C	ACE	non-coding intronic
rs4277404	chr17	61592753	1101	C	T	ACE	non-coding intronic
rs12451328	chr17	61596548	3795	C	A	ACE	non-coding intronic
rs4968591	chr17	61598118	1570	T	C	ACE	non-coding intronic
rs867640	chr17	61601945	3827	T	C	KCNH6	intronic
rs9914151	chr17	61605286	3341	A	C	KCNH6	intronic
rs11655956	chr17	61608705	3419	G	C	KCNH6	intronic
rs11658641	chr17	61610900	2195	G	T	KCNH6	intronic
rs7225568	chr17	61611423	523	T	C	KCNH6	coding

ing was performed with the Sequenom MassARRAY iPLEX Gold platform (Agena Biosciences, San Diego, California) with standard protocols. Genotypes were called using Sequenom's MassARRAY Typer software. As quality controls, four water controls and four duplicated DNA samples were included in a plate. The integrity of control and duplicate sample results was checked during the evaluation process.

1.4. Statistical analyses

LD and association analyses for SNPs were done with a total of 188 Finnish sarcoidosis patients and 150 healthy controls and 139 Czech sarcoidosis patients and 176 controls. The SNPs were excluded if the minor allele frequency was <0.05, individual genotyping call rate was <0.95 and deviation from Hardy-Weinberg equilibrium (HWE) p<0.001. In the Finnish subset, six SNPs failed to meet the minor allele frequency borderline and were excluded from the study leaving a total of 23 SNPs for the final analysis. In the Czech subset, four SNPs did not meet the minor allele frequency borderline leaving a total of seven SNPs for analyses. Pairwise LD (r^2) between genotyped SNPs was conducted using Haploview software (version 3.32) (26). The association analyses between SNPs and disease susceptibility and severity were performed using PLINK software (27). A threshold of 0.05 was used to measure statistical significance. To assess the LD block of disease associated SNP, a SNP Annotation and Proxy Search (SNAP) was utilized (http://www.broadinstitute.org/mpg/snap/ldsearch.php). A threshold of 0.8 was used for pairwise LD.

Chi-square and Fisher's exact test when appropriate were used to assess significant differences in test marker frequencies (elevated S-ACE, I/D polymorphism, *ACE* SNPs) between the groups (all sarcoidosis patients, persistent disease, resolved disease and controls). The results are presented as uncorrected p values, except the *ACE* SNP genotype level analyses were corrected for multiple comparisons by using both False Discovery Rate (FDR) method and Bonferroni correction. A value of p<0.05 was considered statistically significant and odds ratios (ORs) with 95 % confidence intervals (CIs) were utilized to evaluate the strength of the associations. The effect of test markers for sarcoidosis prognosis was analyzed with logistic regression analysis (forward stepwise). The results are presented as uncorrected p values. Statistical tests were done using PASW Statistics (PASW Statistics 18, SPSS Inc.).

Power calculation was assessed to determine the power of Czech sample size to detect the association between the found *ACE* SNP and severity of sarcoidosis. Calculation was done using risk allele frequency of 0.52, prevalence=0.5 (prevalence of good prognosis patients among all sarcoidosis patients), OR=2.03, D′=1, α=0.05, which resulted in 73% power (http://pngu.mgh.harvard.edu/~purcell/gpc/) (Purcell, Cherny & Sham 2003).

2. Results

2.1. SNP rs4343 detects I/D polymorphism

Both PCR based methods and SNP rs4343 genotyping showed consistent results: the G allele was always found in samples with deletion and the A allele with insertion (data not shown). The results confirmed that rs4343 can be used for detecting I/D polymorphism.

2.2. ACE I/D polymorphism and elevated S-ACE activity in disease subgroups

Insertion/deletion polymorphism detected by tag SNP rs4343 did not show significant differences in allelic or phenotype frequencies when compared between all sarcoidosis patients and controls or between disease subgroups (Table 2). Instead, I/D genotypes associated with elevated S-ACE activity; 30.2% of individuals with DD genotype had elevated S-ACE compared to 6.0% with II or ID genotype (p<0.001, OR=6.76 (95%CI 2.68-17.03)), especially in the persistent disease subgroup (43.3% vs. 13.0%, p=0.037, OR=5.10 (95%CI 1.24-20.93), Fig. 1). The elevated S-ACE + DD genotype was found significantly more frequently than the normal S-ACE + II/ID genotype in patients with persistent disease than in patients with resolved disease (13.4% vs. 3.5%, p=0.015, OR=4.44 (95%CI 1.22-16.13)). No association with disease subgroups was found when a dominant model for the D phenotype (DD/ID) and elevated S-ACE levels were used.

Systemic corticosteroid treatment usually lowers the S-ACE activity (28, 29). Out of all patients

Table 2. Allele and genotype frequencies of insertion/deletion (I/D) polymorphism in Finnish sarcoidosis patients and controls and their association between disease subgroups

SNP	All patients	Resolved	Persistent	Controls	Persistent vs. Resolved	
					p	OR
I/D (rs4343)*						
Allele frequency†						
D %	47.8	48.3	47.4	48.7	0.86	1.04
I %	52.2	51.7	52.6	51.3		
Genotype frequency‡						
II n(%)	45 (24.2)	20 (22.5)	25 (25.8)	37 (24.8)		
ID n(%)	88 (47.3)	46 (51.7)	42 (43.3)	71 (47.7)		
DD n(%)	53 (28.5)	23 (25.8)	30 (30.9)	41 (27.5)	0.44	1.29
DD + ID n(%)	141 (75.8)	69 (77.5)	72 (74.2)	112 (75.2)	0.60	1.20

*Variant alleles referred as G=D (deletion) and A=I (insertion)
†Number of subjects: all patients, n=188 (resolved, n=90; persistent, n=98); controls n=150
‡Number of subjects: all patients, n=186 (resolved, n=89; persistent, n=97); controls n=149

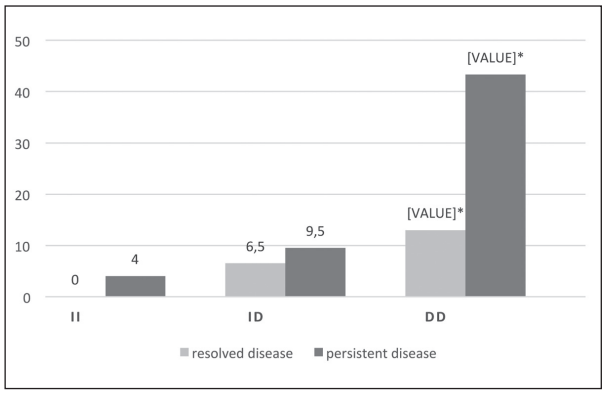

Fig. 1. Frequency (%) of the elevated S-ACE activity (y axis) in relation to insertion/deletion polymorphism genotypes (II, ID, DD) in Finnish resolved and persistent sarcoidosis patients. *χ^2: 43.3% vs. 13.0%, p=0.037, OR=5.10 (95%CI 1.24-20.93)

twenty had received steroid treatment (persistent disease, n=18 and resolved disease, n=2). Five patients in the steroid treated persistent group had elevated S-ACE activity and none in the resolved group. To eliminate possible distortion of the S-ACE activity because of steroid treatment we selected the untreated patients for comparisons (persistent disease, n=79; resolved disease, n=87). The frequencies of elevated S-ACE in relation to I/D genotypes in persistent and resolved subgroups were as follows: II 4.8% vs. 0%; ID 8.3% vs. 6.5%; DD 40.9% vs. 14.3%, respectively. The elevated S-ACE + DD genotype was more common in the persistent subgroup than in the resolved subgroup, but the difference did not reach significance (p=0.088). The distribution of DD vs. DI/II genotypes did not differ between the persistent and resolved subgroups (27.8% vs. 24.1%, p=0.57, respectively). Again, the elevated S-ACE + DD genotype was overrepresented in patients with persistent disease compared to those with resolved disease [11.4 % vs. 3.4%, p=0.048, OR=3.60 (95%CI 0.94-13.81)]. Also in this group no association was found when we compared DD + ID vs. II in relation to elevated S-ACE.

2.3. ACE SNPs in disease subgroups

Allele frequencies of the 23 SNPs of the *ACE* gene region did not differ significantly between all sarcoidosis patients vs. controls, persistent subgroup vs. controls, resolved subgroup vs. controls and persistent vs. resolved subgroups (Supplementary Table 2). In the genotype comparisons, two SNPs, rs9905945 and rs8076157, from the 5' upstream region of the *ACE* gene were significantly associated with the resolved subgroup when compared with the persistent subgroup (Table 3). However, after correcting the analyses for multiple comparisons, the significance did not abide. By logistic regression only rs9905945 was an independently associated marker for the resolved group (p=0.033, OR=2.07, (95%CI 1.06-4.03)). In both disease subgroups SNPs rs9905945 and rs8076157 showed moderate LD with each other, but not with any other SNPs of the *ACE* gene region (Fig. 2). Overall, the LD patterns in the *ACE*

Table 3. Significantly associated SNPs located in the *ACE* gene region and their allele and genotype distributions among Finnish patients with sarcoidosis and healthy controls

Variant		All patients	Resolved	Persistent	Controls	Resolved vs. Persistent	
						p	OR (95% CI)
ACE (promoter)	rs9905945						
	Allele frequency†						
	C %	48.7	52.3	45.3	48.7	0.18	1.32
	T %	51.3	47.7	54.7	51.3		
	Genotype frequency‡						
	TT n(%)	51 (27.4)	18 (20.2)	33 (34.0)	39 (26.2)		
	CT n(%)	91 (48.9)	49 (55.1)	42 (43.3)	76 (51.0)		
	CC n(%)	44 (23.7)	22 (24.7)	22 (22.7)	34 (22.8)	0.744	1.12 (0.57-2.20)
	CC + CT n(%)	135 (72.6)	**71 (79.8)**	**64 (66.0)**	110 (73.8)	**0.035***	2.034 (1.045-3.960)
ACE (promoter)	rs8076157						
	Allele frequency†						
	T %	27.3	31.5	23.4	28.3	0.083	1.5
	C %	72.7	68.5	76.6	71.1		
	Genotype frequency‡						
	CC n(%)	100 (53.7)	41(46.1)	59 (60.8)	81 (54.4)		
	CT n(%)	72 (38.7)	40 (44.9)	32 (33.0)	52 (34.9)		
	TT n(%)	14 (7.5)	8 (9.0)	6 (6.20)	16 (10.7)	0.48	1.48(0.49-4.45)
	TT + CT n(%)	86 (46.2)	**48 (53.9)**	**38 (39.2)**	68 (45.6)	**0.044***	1.818 (1.015-3.256)

†Number of subjects: all patients, n=188 (resolved, n=90; persistent, n=98); controls n=150
‡Number of subjects: all patients, n=186 (resolved, n=89; persistent, n=97); controls n=149
*p value not significant after correcting for multiple comparison.

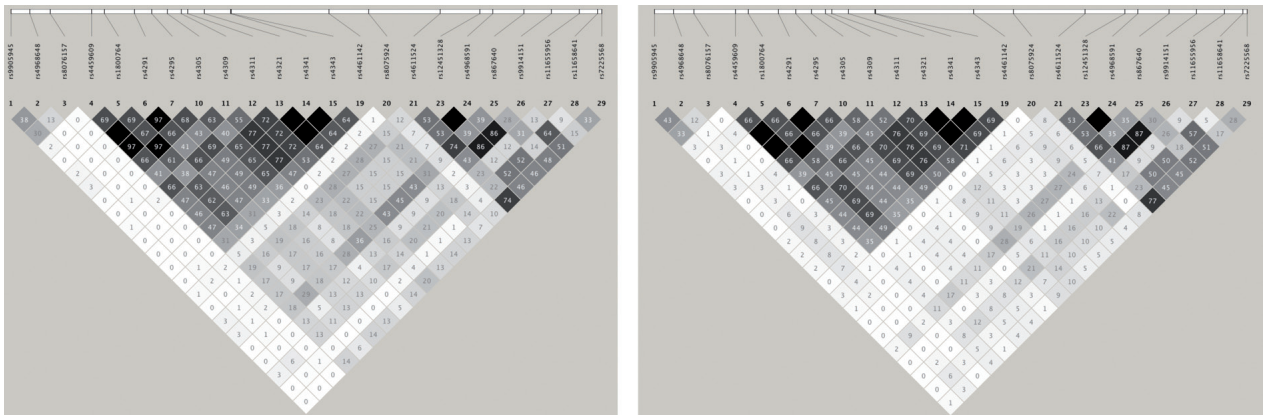

Fig. 2. Pairwise linkage disequilibrium (r^2) pattern of 23 single nucleotide polymorphisms (SNPs) in the *ACE* gene region in Finnish sarcoidosis patients with resolved and persistent sarcoidosis, respectively

gene region showed little difference between disease subgroups (Fig. 2) or between all patients and controls (data not shown). There was no association between rs9905945 and elevated S-ACE activity (data not shown).

2.4. Replication of ACE SNPs

Replication of the *ACE* SNPs was performed using the Czech data set (Supplementary Table 1). Distribution of genotypes and alleles of 7 SNPs did not differ between all sarcoidosis patients vs. controls and persistent vs. resolved patient subgroups (Supplementary Table 3) and the SNP rs9905945 did not show association with disease susceptibility or severity (Supplementary Table 4). Overall, the LD patterns in the *ACE* gene region in persistent and resolved disease groups were similar between the Czech and the Finnish samples (Supplementary Fig. 1). The LD between rs9905945 and the other promoter region SNPs was however not as strong as in the Finnish patients (data not shown).

3. Discussion

Sarcoidosis is a systemic granulomatous disease characterized by epithelioid granulomas in affected organs (30). Although the etiology of the disease is unknown it is thought to involve a complex interplay between genes and external agents. Current understanding is that antigen-presenting cells present peptides in a way that recognition by CD4+ T lymphocytes initiates an inflammatory response resulting in granuloma formation (31). Thus HLA class II genes are likely to be involved in sarcoidosis susceptibility. On the other hand, epithelioid cells in granulomas are the main source of ACE. High S-ACE levels are widely observed in sarcoidosis and are thought to correlate with granuloma mass and sarcoidosis activity (32) making the *ACE* gene as a whole a potential susceptibility factor as well (14).

Compared to changes in chest radiographic findings and lung function tests serum biomarkers have been less satisfactory in the identification of active sarcoidosis (33). Although S-ACE is elevated in some 50% of all newly diagnosed patients, serum levels may be normal in active disease and do not correlate with e.g. chest radiographic findings (34). Similarly, the correlation between the extent of nodular changes and consolidation on high resolution CT and S-ACE is weak (35). Improvements in the sensitivity of S-ACE with the discovery of the I/D polymorphism of the *ACE* gene and the use of genotype-specific reference ranges have been counteracted by a reduction in the already low specificity (36). It is therefore not surprising that opinions have been expressed that measuring S-ACE does not add usefulness to e.g. pulmonary function tests and imaging in the staging and monitoring of sarcoidosis (37).

This study aimed to test the hypothesis whether the *ACE* DD genotype and variants spanning the whole *ACE* gene affect disease outcome in Finnish sarcoidosis patients. We did not demonstrate any association between the *ACE* I/D phenotype or allele frequency and disease susceptibility or severity. Our findings are in contrast with the results of our previous study in Finnish sarcoidosis patients where an association with the DD genotype and poor disease prognosis was found (18). In this current study we assessed the I/D polymorphism by genotyping the SNP rs4343 and by two PCR based methods, another of these utilized in our previous study. All methods gave consistent results, suggesting that this SNP is a tagging SNP for the I/D polymorphism in the Finnish population and that the inconsistent results with the previous study are not due the different genotyping methods, but probably a result of the small number of subjects in the first study (sarcoidosis patients, n=59; controls, n=70).

Similar results of no association between disease susceptibility or severity and the I/D polymorphism have been previously described (13, 38-40). In these studies the S-ACE activity was shown to be affected by insertion/deletion polymorphism showing the lowest values in II genotypes and the highest in the DD genotype. This was also evident in our study. The S-ACE activity increased according to the D genotype in the resolved patient group, but even more evidently in the persistent disease group. It should be noted that the S-ACE activity was measured at the point of study enrollment when at least five years had passed since the time of diagnosis. It is therefore logical that patients with persistent disease have more elevated S-ACE values. Still, our study suggests that the DD genotype has an influence on the S-ACE levels, at least with prolonged high activity.

To our knowledge the effect of the other *ACE* variants besides I/D polymorphism has been rarely studied on sarcoidosis susceptibility or severity. Upstream and downstream variations of the gene have been shown to be in LD with the I/D polymorphism probably indicating that the association between the I/D polymorphism and sarcoidosis is representative for the entire *ACE* gene (19). In this study, we did not find association between other *ACE* variants and disease susceptibility or severity in Finnish sarcoidosis patients. However, the C variant of SNP rs9905945 located in the 5' upstream region of the *ACE* gene showed moderate association with good prognosis. The SNP rs9905945 does not seem to be in LD with any of the SNPs in the *ACE* region, including the I/D polymorphism. According to the data form SNAP database the SNP rs9905945 is in LD with SNPs located further from the *ACE* gene region probably suggesting that the association is not related to the *ACE* gene itself. Main annotation for SNP rs9905945 is in *MSI2* (*Musashi RNA binding protein 2*) which encodes a protein containing two conserved tandem RNA recognition motifs. Similar proteins in other species function as RNA-binding proteins and play central roles in posttranscriptional

gene regulation. Currently there is no data available about functional properties of the SNP rs9905945.

In the replication cohort of Czech patients the association between sarcoidosis and SNP rs9905945 was not detected, although the overall LD structure in the *ACE* region was similar to the Finnish population. The Czech and Finnish patients might have divergent LD structures further from the *ACE* region explaining this non-replication. Also the genetic power in Czech sample size may have not been sufficient enough to detect the observed moderate association. In Finnish sarcoidosis patients the association was in its entirety moderate, leading a possibility of false association. Further investigation in expanded cohorts is, therefore, needed to test this hypothesis.

There are some limitations for this study. The sarcoidosis patients were recruited all over Finland and the S-ACE activity was analyzed in different laboratories. Due to different laboratory methods, the S-ACE values could be classified only as elevated S-ACE activity or normal S-ACE activity. Therefore an overall variance of the S-ACE levels between each I/D genotype could not been assessed. Also the effect of the I/D polymorphism and the S-ACE levels for disease susceptibility could not be investigated because of the lack of S-ACE data in the control population. Due to the rather high frequency of the rs9905945 C allele in both patient subgroups the effect of rs9905945 should be determined in a larger data set.

In conclusion, our results indicate that the *ACE* I/D polymorphism does not play a major role in explaining the clinical characteristics of Finnish sarcoidosis patients. Similar results were found in relation to the other investigated *ACE* polymorphisms. We discovered a novel SNP upstream from the *ACE* gene region with prospective association with favorable disease prognosis, however the association was only moderate and did not replicate in the Czech population. In conclusion, it seems that variants in the *ACE* gene do not considerably influence the course of disease in Finnish sarcoidosis patients.

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SNP Annotation and Proxy Search (SNAP) http://www.broadinstitute.org/mpg/snap/ldsearch.php

Appendix. Supplementary data

Supplementary Table 1. List of single nucleotide polymorphisms (SNPs) genotyped from the Czech samples. SNPs marked in grey did not meet genotyping criteria (minor allele frequency >0.05)

SNP	Chromosome	Position	Allele 1	Allele 2	Gene	Predicted function
s9905945	chr17	61539056	C	T		
rs4968648	chr17	61541379	G	A		
rs8076157	chr17	61543861	T	C		
rs4459609	chr17	61548948	C	A		
rs1800764	chr17	61550529	C	T		
rs4291	chr17	61554194	T	A	ACE	5' upstream
rs4295	chr17	61556298	C	G	ACE	intronic
rs4305	chr17	61558229	A	G	ACE	intronic
rs4309	chr17	61559923	C	T	ACE	coding
rs4311	chr17	61560763	T	C	ACE	5' upstream
rs4343	chr17	61566031	G	A	ACE	coding

Supplementary Table 2. Allele frequencies of single nucleotide polymorphisms (SNPs) in the *ACE* gene region and their association between all Finnish sarcoidosis patients and healthy controls and between disease subgroups

SNP	All patients[a]	Resolved[a]	Persistent[a]	Controls[a]	All patients vs. Controls		Resolved vs. Persistent	
	%	%	%	%	P	OR	P	OR
rs9905945	48.7	52.3	45.3	48.7	1.00	1.00	0.18	1.32
rs4968648	31.0	27.8	34.0	32.5	0.68	0.93	0.20	0.75
rs8076157	27.3	31.5	23.4	28.3	0.77	0.95	0.083	1.50
rs4459609	36.8	37.1	36.5	32.3	0.23	1.22	0.90	1.03
rs1800764	46.0	46.1	45.8	44.0	0.61	1.08	0.96	1.01
rs4291	36.8	37.1	36.5	32.3	0.23	1.22	0.90	1.03
rs4295	36.5	36.5	36.5	32.3	0.26	1.20	0.99	1.00
rs4305	45.7	45.5	45.8	44.3	0.73	1.06	0.95	0.99
rs4309	41.9	43.3	40.6	42.0	0.98	1.00	0.61	1.11
rs4311	43.8	43.8	43.8	40.0	0.32	1.17	0.99	1.00
rs4321	48.1	48.3	47.9	48.7	0.88	0.98	0.94	1.02
rs4341	47.8	48.3	47.4	48.7	0.83	0.97	0.86	1.04
rs4343	47.8	48.3	47.4	48.7	0.83	0.97	0.86	1.04
rs4461142	46.2	43.8	48.4	44.3	0.63	1.08	0.37	0.83
rs8075924	10.3	11.4	9.4	14.2	0.13	0.70	0.53	1.24
rs4611524	47.6	50.0	45.3	49.7	0.59	0.92	0.37	1.21
rs12451328	37.0	34.8	39.1	32.0	0.17	1.25	0.40	0.83
rs4968591	37.0	34.8	39.1	32.3	0.21	1.23	0.40	0.83
rs867640	38.9	42.7	35.4	40.3	0.71	0.94	0.15	1.36
rs9914151	38.7	37.1	40.1	34.0	0.21	1.22	0.55	0.88
rs11655956	15.7	19.1	12.5	12.7	0.27	1.28	0.081	1.65
rs11658641	27.6	27.5	27.6	27.0	0.87	1.03	0.99	1.00
rs7225568	45.7	46.6	44.8	47.7	0.61	0.92	0.72	1.08

[a]Number of subjects: all patients, n=188 (resolved, n=90; persistent, n=98); controls n=150

Supplementary Table 3. Allele frequencies of single nucleotide polymorphisms (SNPs) in the *ACE* gene region and their association between all Czech sarcoidoisis patients and healthy controls and between disease subgroups

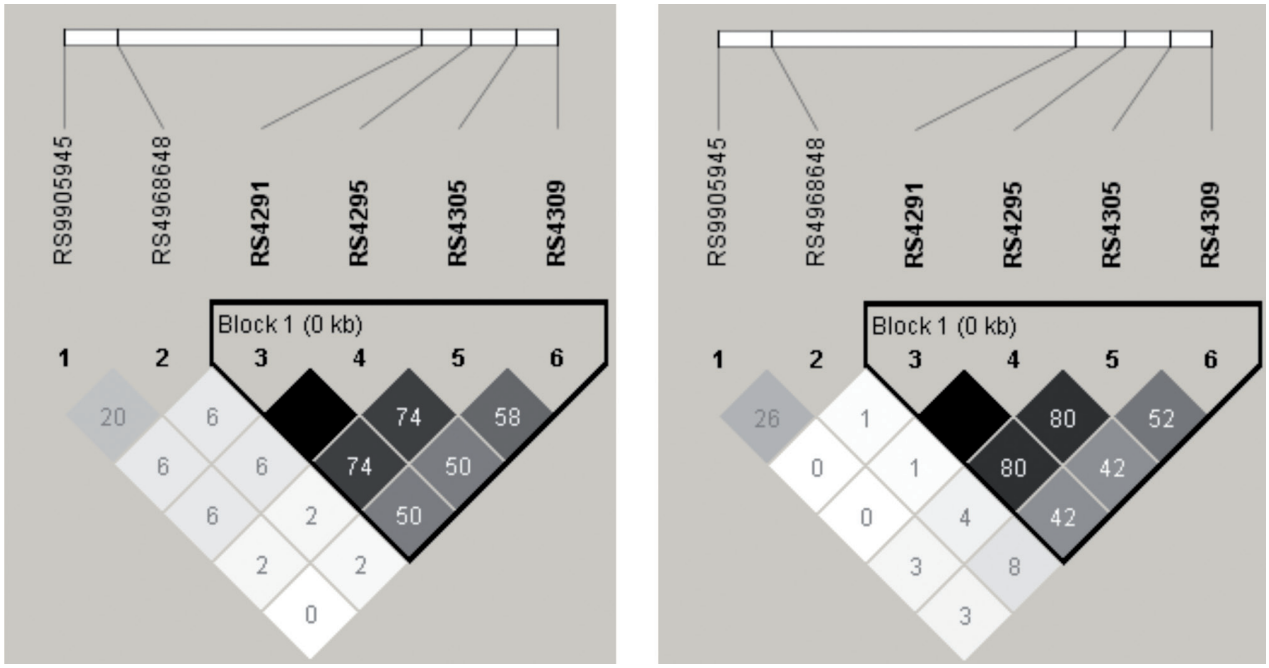
SNP	All patients[a]	Resolved[a]	Persistent[a]	Controls[a]	All patients vs. Controls		Persistent vs. Resolved	
	%	%	%	%	P	OR	P	OR
rs9905945	41.4	38.3	42.9	47.2	0.39	1.28	0.47	1.23
rs4968648	27.0	24.5	28.3	26.1	0.86	0.95	0.63	1.17
rs4291	63.3	58.5	45.7	64.6	0.76	1.09	0.07	0.59
rs4295	63.3	58.5	66.3	64.6	0.76	1.09	0.31	1.35
rs4305	41.0	45.7	38.6	43.5	0.67	1.13	0.32	0.75
rs4309	53.6	56.4	52.2	53.7	1.0	1.0	0.57	0.85
rs4341	61.2	58.5	62.5	61.6	0.89	1.04	0.56	1.18

[a]Number of subjects: all patients, n=139 (resolved, n=47; persistent, n=92); controls n=176

Supplementary Table 4. Significantly associated SNP located in the *ACE* gene region and their distributions among Czech and combined (Finnish + Czech) patients with sarcoidosis and healthy controls

Variant		All patients	Resolved	Persistent	Controls	Resolved vs. Persistent	
						p	OR (95% CI)
ACE (promoter)	rs9905945 Genotypes						
	Czech samples[a]						
	TT n(%)	47 (33.8)	17 (36.2)	30 (32.6)	50 (28.4)		
	CT n(%)	69 (49.6)	24 (51.1)	45 (48.9)	86 (48.9)		
	CC n(%)	23 (16.5)	6 (12.8)	17 (18.5)	40 (22.7)	0.475	0.65 (0.24-1.76)
	CC + CT n(%)	92 (66.2)	30 (63.8)	62 (67.4)	126 (71.6)	0.707	0.85 (0.41-1.79)
	Combined[b]						
	TT n(%)	98 (30.2)	35 (25.7)	63 (33.3)	89 (27.4)		
	CT n(%)	160 (49.2)	73 (53.7)	87 (46.0)	162 (49.8)		
	CC n(%)	67 (20.6)	28 (20.6)	39 (20.6)	74 (22.8)	1.00	1.00 (0.58-1.72)
	CC + CT n(%)	227 (69.8)	101 (70.3)	126 (66.7)	236 (72.6)	0.144	1.44 (0.88-2.35)

[a]Number of subjects: all patients, n=139 (resolved, n=47; persistent, n=92); controls, n=176
[b]Number of subjects (Finnish + Czech): all patients, n=325 (resolved, n=136; persistent, n=189); controls, n=325



Supplementary Fig 1. Pairwise linkage disequilibrium (r^2) pattern of 7 replicated single nucleotide polymorphisms (SNPs) in the *ACE* gene region in Czech sarcoidosis patients with resolved and persistent sarcoidosis, respectively