

## The angiotensin-converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients

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*The angiotensin-converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients. A. Pietinalho, K. Furuya, E. Yamaguchi, Y. Kawakami, O. Selroos. ©ERS Journals Ltd 1999.*

**ABSTRACT:** Angiotensin-converting enzyme (ACE) genotypes may reflect prognosis in sarcoidosis.

They were determined in 59 Finnish sarcoidosis patients and 70 healthy control subjects. The prognosis of the sarcoidosis patients was determined after follow-up for 1, 2, 3, 5 and >5 yrs and classified as good (normal chest radiograph and lung function, no signs of extrapulmonary disease activity within 2 yrs from diagnosis), intermediate (neither good nor poor) or poor (persisting unstable pulmonary infiltrates, vital capacity and diffusing capacity of the lung for carbon monoxide <50% predicted and/or extrapulmonary disease activity after >5 yrs follow-up).

The DD, ID and II genotypes were found in 31 and 27%, in 54 and 49%, and in 15 and 24% of patients and control subjects respectively. The odds ratio (DD+ID to II) was 1.45 (95% confidence interval 0.60–3.49). The D allele was found more often in patients (58%) and in control subjects (51%) than the I allele but the difference was not statistically significant. Statistically significantly more patients with the DD genotype had a poor prognosis compared with patients with II homozygotes and ID heterozygotes. Among 11 patients with Löfgren's syndrome (bilateral hilar lymphadenopathy and erythema nodosum), four had the DD genotype. Three of these patients had a prognosis despite presenting a clinical picture usually associated with a good prognosis.

The angiotensin-converting enzyme genotype may be a prognostic marker in sarcoidosis and larger studies are warranted to define its clinical utility.

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The angiotensin-converting enzyme (ACE) gene has a polymorphism based on the presence or absence of a nonsense deoxyribonucleic acid (DNA) fragment [1]. The polymorphism is located in intron 16, so the ACE protein does not differ due to genotype, but the polymorphism accounts for 47% of the total enzyme level [2]. The genotype is classified into three types: insertion homozygotes (II), deletion homozygotes (DD), and heterozygotes (ID). Serum ACE activity has been found to be significantly higher in DD individuals than in II individuals. This applies to both healthy control subjects and patients with sarcoidosis [2–4]. In Caucasians, the D allele appears to be dominant. I/D ratios of 0.41:0.59 [1], 0.45:0.55 [5] and 0.40:0.60 [3] have been reported. In Japanese subjects, the I allele is dominant, with I/D ratios of 0.67:0.33 in control subjects and 0.61:0.39 in sarcoidosis patients reported by FURUYA *et al.* [4], and 0.65:0.35 in controls and 0.62:0.38 in sarcoidosis patients by TOMITA *et al.* [2]. As no significant difference has been found in the allele distribution between controls and sarcoidosis patients, a particular ACE polymorphism does not predispose to the disease. From an epidemiological point of view, however, it is interesting that predominance of the I allele occurs in countries such as Japan where the prevalence of sarcoidosis is lower than in Western countries. In prior

studies, a significantly higher prevalence of sarcoidosis was found in Finland than in Hokkaido, Japan [6].

Japanese sarcoidosis patients have a significantly better prognosis than Finnish patients [7]. There is some indication that Japanese patients with the ACE DD genotype have a more prolonged disease than patients with other genotypes [2]. As the DD genotype dominates in Caucasians, it was of interest to determine the genotypes in a Finnish sarcoidosis population and to evaluate and compare the prognosis of patients with the three genotypes.

### Material and methods

Samples of whole blood were drawn from 59 consecutive Finnish sarcoidosis patients visiting the outpatient department of the hospital for scheduled visit and 70 healthy control subjects. They were all known sarcoidosis patients who had been followed-up for 1–23 yrs. The blood samples were stored at -70°C until shipped to Sapporo, Japan, for analysis.

The patient characteristics at diagnosis, in respect to ACE genotypes, are shown in table 1. There were 42 female and 17 male patients. All patients had chest radiographic changes. A total of 27 patients had radiographic stage I lesions, and 20 patients had stage II

Table 1. – Patient characteristics

|                          | Genotype |    |    | Total |
|--------------------------|----------|----|----|-------|
|                          | II       | ID | DD |       |
| Female                   | 7        | 23 | 12 | 42    |
| Male                     | 2        | 9  | 6  | 17    |
| Biopsy confirmed         | 6        | 20 | 15 | 41    |
| Steroid treatment        | 5        | 19 | 11 | 35    |
| Pulmonary findings:      |          |    |    |       |
| Stage 0                  | 0        | 1  | 1  | 2     |
| Stage I                  | 5        | 17 | 5  | 27    |
| Stage II                 | 2        | 10 | 8  | 20    |
| Stage III                | 2        | 4  | 3  | 9     |
| Extrapulmonary findings: |          |    |    |       |
| Erythema nodosum         | 3        | 4  | 4  | 11    |
| Peripheral lymph node    | 0        | 7  | 2  | 9     |
| Skin                     | 2        | 6  | 3  | 11    |
| Eyes                     | 1        | 7  | 2  | 10    |
| Other*                   | 4        | 7  | 6  | 17    |
| Hypercalcaemia           | 1        | 3  | 1  | 5     |

Data presented as number of patients. \*: including spleen, liver, kidney, heart, central nervous system and parotid gland.

lesions. A total of 31 patients (53%) had extrapulmonary sarcoidosis other than erythema nodosum (EN), which occurred in 11 patients. In one patient with Löfgren's syndrome (EN, arthralgia and a stage I radiographic finding), no biopsy support was obtained. The patient recovered spontaneously within 1 yr after diagnosis. There appeared to be no important differences regarding disease manifestations between the three ACE genotype subgroups.

#### Detection of ACE gene polymorphism

A 287 base pair I/D polymorphism in intron 16 of the ACE gene was examined by polymerase chain reaction (PCR) as previously described [4]. Briefly, two primers, sense oligo 5'-CTGGAGACCACTCCCATCCTTCT-3' and antisense oligo 5'-GATGTGGCCATCACATTCGT-CAGAT-3', were synthesized to amplify the polymorphic fragment. Reactions were performed with 10 pmol of each primer in a final volume of 50 µL containing 100 ng of genomic DNA, 3 mM MgCl<sub>2</sub>, 50 mM KCl, 10 mM Tris-HCl, (pH 8.4), 0.1 mg·mL<sup>-1</sup> gelatin<sup>-1</sup>, 0.5 mM of each deoxynucleotide triphosphate (dNTP), 1 unit of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). The DNA was amplified for 30 cycles as previously described [8]. The PCR products were subjected to electrophoresis in agarose gels and visualized by ethidium bromide staining. Each allele and genotype was determined by the size of bands as described previously [9].

#### Determination of prognosis of sarcoidosis

The prognosis was evaluated after observation periods of 1, 2, 3, 5 and >5 yrs after diagnosis. The prognosis was classified as good when the chest radiograph had become normal within 2 yrs, the lung function was normal (vital capacity (VC) and diffusing capacity of lung for carbon monoxide (DLCO)), no signs of extrapulmonary sarcoidosis were detectable, and the biochemical markers of

sarcoidosis activity (serum ACE, lysozyme and calcium, as well as urinary calcium levels) were normal. The prognosis was classified as poor when after 5 yrs of follow-up, persisting, unstable chest radiographic infiltrates, impaired lung function (VC and DLCO, <50% predicted normal values) and/or active extrapulmonary disease in combination with increased levels of one or more biochemical markers of sarcoidosis activity were found. The prognosis of patients who did not fulfil the above criteria was classified as intermediate. These patients might have obtained a normal status between 2–5 yrs of follow-up or have had remaining chest radiographic infiltrates with borderline signs of disease activity.

#### Statistical methods

The difference in allele and genotype frequencies between control subjects and sarcoidosis patients was tested by Chi-square analysis. Odds ratios were calculated to estimate the relative risk of sarcoidosis and the analyses were carried out by using logistic regression models adjusted for sex and age.

The differences in frequency distribution of patients with good, intermediate or poor prognosis between the genotype groups were tested by Chi-square analysis.

## Results

#### ACE genotype frequencies

The ACE genotype frequencies are shown in table 2. The genotype frequencies were not statistically different between sarcoidosis patients and control subjects: odds ratio 1.45 (95% confidence interval (CI) 0.60–3.49). The frequency of the D allele was higher than that of the I allele: 0.51:0.49 in control subjects and 0.58:0.42 in sarcoidosis patients. The difference was not statistically significant ( $p=0.32$ ).

#### Prognosis and ACE genotypes

The prognosis of the sarcoidosis patients is shown in table 3. Only two patients of 18 with the DD genotype had a good prognosis defined as described above and three had a prognosis classified as intermediate. Patients with a good prognosis were compared with the rest of the patients (poor and intermediate prognosis). A good prognosis was significantly less frequent among patients with the DD genotype than among those with the II and ID genotypes ( $p<0.05$ ). Similarly, 13 of the 18 DD patients had a poor prognosis. This finding was also statistically significantly different from the results in patients with II or ID genotypes when the patients with a poor prognosis were compared with those with a good or intermediate prognosis ( $p<0.01$ ).

#### Erythema nodosum

A total of 11 patients (four DD individuals, four ID, and three II) had had EN, which is usually described as a sign of good prognosis. All three patients with the II genotype

Table 2. – Angiotensin-converting enzyme genotype and allele frequencies in sarcoidosis patients and controls

|                    | Females   |          | Males     |          | Total     |          |
|--------------------|-----------|----------|-----------|----------|-----------|----------|
|                    | Patients  | Controls | Patients  | Controls | Patients  | Controls |
| Genotype frequency |           |          |           |          |           |          |
| DD n(%)            | 12 (29)   | 14 (30)  | 6 (38)    | 5 (22)   | 18 (31)   | 19 (27)  |
| ID n(%)            | 23 (55)   | 23 (49)  | 9 (56)    | 11 (48)  | 32 (54)   | 34 (49)  |
| II n(%)            | 7 (17)    | 10 (21)  | 2 (13)    | 7 (30)   | 9 (15)    | 17 (24)  |
| Odds ratio*        | 1.17      |          | 3.08      |          | 1.45      |          |
| 95% CI             | 0.41–3.32 |          | 0.50–18.9 |          | 0.60–3.49 |          |
| p-values           | 0.77      |          | 0.22      |          | 0.41      |          |
| Allele frequency   |           |          |           |          |           |          |
| D                  | 0.56      | 0.54     | 0.625     | 0.46     | 0.58      | 0.51     |
| I                  | 0.44      | 0.46     | 0.375     | 0.54     | 0.42      | 0.49     |
| p-values           | 0.82      |          | 0.14      |          | 0.32      |          |

\*: Ratio of DD+ID to II. CI: confidence interval

and three of the four with the ID genotype had a good prognosis, whereas three of four EN patients with the DD genotype had a poor prognosis. These three patients with initial stage I (one patient) or stage II radiographic (two patients) findings have been followed for 6, 7 and 19 yrs, respectively. They have respectively developed 1) splenomegaly and skin lesions, 2) lung fibrosis, hypercalcaemia/hypercalciuria and liver involvement, and 3) lung fibrosis, splenomegaly, renal insufficiency and facial palsy. In all three patients the serum ACE activity has remained significantly elevated.

The difference in prognosis between the DD and the II and ID patients with EN was statistically significant ( $p < 0.05$ ).

### Discussion

This study in 59 Finnish sarcoidosis patients and 70 healthy control subjects has confirmed earlier reports showing a higher frequency of the D allele of the ACE gene than of the I allele in Caucasian subjects [1, 3, 5]. This is in contrast to the findings in Japanese subjects [2, 4]. Also, as in previous studies, a specific ACE genotype was not found to represent a risk factor for developing sarcoidosis.

An important finding in the present study was the difference in prognosis between sarcoidosis patients with different ACE genotypes. Patients with the DD genotype had a significantly poorer prognosis than the other patients. This was even true of patients with EN, which has been regarded as a sign of a very good prognosis [10], although exceptions have been reported [11].

Earlier studies have shown lower serum ACE activity in II genotype patients than in DD genotype patients, with the ID genotype patients having values between the two other

groups [2, 4]. In this series of Finnish patients, this finding cannot be proved or disproved, as the measurements of serum ACE in the present patients at the time of diagnosis were performed in different laboratories and by using slightly different methods. The measurements at the time of the ACE gene determinations were known, but as 35 patients were receiving or had received treatment with corticosteroids, these serum ACE values do not give information about possible differences in activity levels between Finnish sarcoidosis patients with different ACE genotypes.

A prior study has shown that Finnish sarcoidosis patients in general have a much less favourable prognosis than the patients seen in Hokkaido, Japan [7]. Possible explanations for this difference after excluding differences in diagnostic procedures, in indications for starting treatment with corticosteroids and in monitoring of disease activity during follow-up have been speculated upon. It has previously, however, been demonstrated that differences in the clinical picture of sarcoidosis between Finnish and Japanese sarcoidosis patients exist [12]. The present finding of a high prevalence of ACE DD genotype in Finnish patients (58% as compared to 38% [2] and to 39% [4] in Japanese patients) gives a further possible explanation for the difference in prognosis, as the DD genotype was significantly associated with a poorer prognosis than the II and ID ACE genotypes. Although less frequent than with the II/ID ACE genotype, a tendency towards a more prolonged disease activity with the DD genotype has been reported in Japanese patients [2].

Determination of the angiotensin-converting enzyme genotype may be a prognostic marker in sarcoidosis. However, much larger studies are required in order to determine the possible clinical usefulness of these determinations.

Table 3. – Prognosis according to angiotensin-converting enzyme genotype

| Prognosis    | Genotype |    |    |
|--------------|----------|----|----|
|              | II       | ID | DD |
| Good         | 4        | 12 | 2  |
| Intermediate | 4        | 8  | 3  |
| Poor         | 1        | 12 | 13 |
| Total        | 9        | 32 | 18 |

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