Genotyping increases the yield of angiotensin-converting enzyme in sarcoidosis – a systematic review

Andreas Fløe¹, Hans Jürgen Hoffmann¹, Peter H. Nissen², Holger Jon Møller² & Ole Hilberg¹

ABSTRACT

INTRODUCTION: The diagnosis of sarcoidosis is challenging and involves radiological, clinical and paraclinical evaluation, the latter including the measurement of serum angiotensin-converting enzyme activity (s-ACE), which is elevated in about 60% of sarcoidosis patients. The normal inter-individual biological variation of s-ACE is large. Approximately 50% of the variation is due to a genomic insertion/deletion (I/D) polymorphism in the ACE gene.

METHODS: We searched the MEDLINE library for articles presenting genotype-based reference intervals for s-ACE in healthy people. We summarised the results as weighted mean DD/II ratios of s-ACE. We also summarised the presented frequencies of the genotypes.

RESULTS: We identified nine studies presenting genotype-based reference intervals. All studies found a significant difference between mean s-ACE in the three genotype groups DD, ID and II. The mean DD/II ratio was 1.85 (range: 1.79-1.92) for all studies, 2.01 (1.92-2.10) for Caucasians and 1.64 (1.55-1.73) for Asians. The median frequencies of genotypes among Caucasians were 23% II, 45% ID and 30% DD, and 45% II, 49% ID and 14% DD among Asians.

CONCLUSION: Genotyping for the I/D polymorphism increases the benefit of s-ACE since all studies found significantly different levels between genotype groups in healthy subjects. Genotyping is of special value if s-ACE is between the upper 97.5 percentile for genotype II and DD since values in this interval are at risk of being misclassified. Due to assay variation, genotype-specific reference levels should be verified locally.

Sarcoidosis is an inflammatory, granulomatous disease. Its pathogenesis is unknown, but probably involves genetic predisposition as well as external factors [1]. Approximately 500 new cases are diagnosed in Denmark annually. Diagnosing sarcoidosis is challenging and includes radiological changes, clinical manifestations and paraclinical findings, including measurement of serum angiotensin-converting enzyme activity (s-ACE, peptidyl-peptidase A). ACE has a number of metabolic effects; most notably it catalyses the modification of angiotensin I to angiotensin II, a potent vasoconstrictor [2] and inactivates bradykinin through the kallikrein-kininogen system [3]. It is also a potent pro-inflammatory modulator [4] secreted by activated cells of the monocyte-macrophage cell lineages, which are crucial in the process of granuloma formation. S-ACE is elevated in about 60% of sarcoidosis patients [5], but also in other granulomatous diseases like Gaucher’s disease and tuberculosis [6]. Though the level of s-ACE reflects the mass of granuloma in the body [7], the clinical use of s-ACE in monitoring disease activity is controversial, and recommendations differ between guidelines.

The activity of ACE can be measured by enzyme kinetic methods which most commonly utilise the polypeptide FAPGG (furyl-acryloyl-phenylalanyl-glycyl-glycine), which acts as a synthetic substrate for ACE. The degradation of FAPGG to FAP is visualised by a changed absorption spectrum by spectrophotometry [8]. Several commercial kits for automatic analysis are available.

The normal level of ACE depends on genetic variation in the ACE gene. In intron 16, a common insertion/deletion polymorphism, varying in a 287 base pair sequence, is of importance [9]. The genotypes are termed DD (homozygote for deletion), ID (heterozygote) and II (homozygote for insertion).

The I/D polymorphism is responsible for almost half of the biological variation in s-ACE among healthy individuals [10], s-ACE being highest in individuals carrying the genotype DD and lowest in genotype II.

Analysis of the genotype was previously performed by restriction fragment length polymorphism (RFLP) [9], which has now been replaced by PCR-based methods for identification of the I and D alleles [11] and most recently by high-resolution melting (HRM) technique [12].

Since the I/D polymorphism impacts the normal level of s-ACE, we aimed to summarise current evidence for genotype-based differences in mean values of s-ACE in different ethnic populations.

METHODS

We used PubMed to search the MEDLINE library for articles providing genotype-based reference intervals of s-ACE until June 2013. We applied the following search terms: “sarcoidosis, pulmonary” (Mesh) AND “peptidyl-dipeptidase A” (Mesh), and “sarcoidosis” AND “ace” AND “genotype” (free text search). We restricted the search to articles in English, German and Danish. No limits were set regarding entry year.

From the studies selected, genotype-based mean
values of s-ACE and standard deviations were obtained, as well as size and ethnicity of the study populations. Between-laboratory and between-assay variation in measurement of s-ACE is substantial [8] and, furthermore, results were reported in different units between studies. A quantitative meta-analysis of the mean s-ACE levels would therefore not provide useful information for comparing the genotype groups. Instead, we used ln-transformed mean values of s-ACE from each study for the groups II, ID and DD to calculate ratios of s-ACE between the groups. These ratios (II/DD, II/ID and ID/DD) do not provide clinically meaningful information in themselves, but they serve to evaluate whether the differences between the groups are significant. This is the case if the confidence interval does not include the value one.

We summarised II/DD, II/ID and ID/DD ratios as weighted mean values as the sample sizes varied considerably. Data were expressed as means and two-sided 95% confidence intervals. Furthermore, we calculated ethnicity-stratified, weighted estimates of the II/DD ratio.

Finally, we summarised genotype frequencies from studies in which such data were reported, and ethnicity-grouped median values were obtained.

Where applicable, the review process complied with the Prisma guidelines [13].

Ethics approval
The review included only data from prior studies. Therefore, ethics approval was not needed. All included studies documented that appropriate ethics approvals had been obtained.

### TABLE 1

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Ethnicity</th>
<th>Assay for s-ACE</th>
<th>Genotyping technique</th>
<th>Unit</th>
<th>lower</th>
<th>upper</th>
<th>mean</th>
<th>lower</th>
<th>upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furuya et al [16]</td>
<td>341</td>
<td>Asian</td>
<td>ACE Color, Fujirebio Inc, Tokyo (colorimetric)</td>
<td>PCR and agarose electrophoresis</td>
<td>IU/l</td>
<td>7.6</td>
<td>24.2</td>
<td>11.8</td>
<td>6.8</td>
<td>18.2</td>
</tr>
<tr>
<td>Kruit et al [17]</td>
<td>200</td>
<td>Caucasian</td>
<td>Bühlmann ACE kinetic test, Bühlmann Laboratories AG, CH</td>
<td>Real-time PCR</td>
<td>U/l</td>
<td>–</td>
<td>–</td>
<td>25.9</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Sharma et al [19]</td>
<td>146</td>
<td>Caucasian</td>
<td>–</td>
<td>–</td>
<td>U/l</td>
<td>17.6</td>
<td>4.6</td>
<td>30.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruprecht et al [18]</td>
<td>262</td>
<td>Caucasian</td>
<td>Bühlmann ACE kinetic test, Bühlmann Laboratories AG, CH</td>
<td>PCR and agarose electrophoresis</td>
<td>U/l</td>
<td>15</td>
<td>80.9</td>
<td>32.2</td>
<td>13.7</td>
<td>50.7</td>
</tr>
<tr>
<td>Rigat et al [9]</td>
<td>80</td>
<td>Caucasian</td>
<td>Radio-immunossay</td>
<td>RFLP</td>
<td>µg/l</td>
<td>–</td>
<td>–</td>
<td>299.3</td>
<td>220.5</td>
<td>397</td>
</tr>
<tr>
<td>Biller et al [14]</td>
<td>159</td>
<td>Caucasian</td>
<td>Bühlmann ACE kinetic test, Bühlmann Laboratories AG, CH</td>
<td>Real-time PCR</td>
<td>U/l</td>
<td>12</td>
<td>82</td>
<td>34.8</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>Assay 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159</td>
<td>Caucasian</td>
<td>Trinity Biotech, Bray, Ireland (kinetic test)</td>
<td>Real-time PCR</td>
<td>U/l</td>
<td>7</td>
<td>44</td>
<td>25.4</td>
<td>7</td>
<td>44</td>
</tr>
<tr>
<td>Nissen et al [12]</td>
<td>400</td>
<td>Caucasian</td>
<td>Infinity ACE, Thermo Fischer Scientific, MA, USA</td>
<td>High-resolution melting</td>
<td>U/l</td>
<td>12</td>
<td>60</td>
<td>21.3</td>
<td>6.5</td>
<td>36.1</td>
</tr>
</tbody>
</table>

DD = homozygous for deletion; ID = heterozygous; II = homozygous for insertion; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; s-ACE = serum angiotensin-converting enzyme activity.

<sup>a</sup> 95% CI constructed by the authors from ln-transformed mean value and SD.

<sup>b</sup> s-ACE was measured with two individual assays.

### FIGURE 1

Flow-chart demonstrating the selection of studies. Adopted in revision from Prisma 2009.
RESULTS

The study selection process is outlined in Figure 1. We identified 102 journal articles. By review of title and abstract, 12 articles were relevant for this analysis. Seven articles [14–20] presented new genotype-based reference intervals for s-ACE based on genotyping and s-ACE measurements in healthy individuals. Furthermore, one article [9] presenting genotype-based reference intervals was identified from reference lists and, in addition, data from a recent Danish study [12] were included. The nine studies represented 2,052 healthy individuals. Genotype-based mean values of s-ACE, and standard deviations for all nine studies are shown in Table 1. One study [14] measured ACE activity with two assays. These are provided individually in Table 1.

All studies found significantly different levels of s-ACE between genotype groups, with DD having the highest mean ACE value, II having the lowest mean ACE value and ID having intermediate values. The distribution of ratios of s-ACE (DD/II, DD/ID and ID/II) is shown in Figure 2. The weighted mean DD/II ratio was 1.85 (range: 1.79–1.92) for all studies, 2.01 (1.92–2.10) for Caucasians and 1.64 (1.55–1.73) for Asians. The mean DD/ID ratio and ID/II ratio were both significantly different from one. Therefore, the mean s-ACE was significantly higher for the DD genotype than for the ID genotype, which was again significantly higher than that for the II genotype.

Two studies [16, 20] provided genotyping and s-ACE data for 310 sarcoidosis patients. These are shown in Table 2. Weighted mean DD/II ratio was 1.45 (1.30–1.62) for these patients. Severity of sarcoidosis was indicated roentgenologically ad modum DeRemee, but genotype-based s-ACE levels were not stratified for roentgenologic disease stage.

All included papers documented that none of the participants were being treated with ACE inhibitors. Frequency of I/D genotypes was reported in eight of nine study populations. These are shown in Table 3. When adjusting for ethnicity, the mean frequency of I/D genotypes among Caucasians was 27.6% II, 45.7% ID and 26.7% DD, while the mean frequencies among two Asian studies was 36.1% II, 49.9% ID and 14.0% DD.

DISCUSSION

This review revealed that significant differences of s-ACE between I/D genotype groups were seen in all included studies. On this basis, it seems rational to recommend genotyping of patients if the value of s-ACE is considered part of the diagnostic process for sarcoidosis or for monitoring disease activity in confirmed cases. It is important to note, though, that the benefit of I/D genotyping is primarily derived from the significant differences of s-ACE.
between the genotypes in healthy people. In this analysis, two studies supported that genotyping will also improve the yield of s-ACE in sarcoidosis patients, but more clinical studies are needed to clearly confirm this finding.

The mean s-ACE level for the genotype DD is almost twice that of genotype II. Variance analyses of previous data have shown that almost 50% of the normal variation in s-ACE in healthy people is attributable to the polymorphism [9, 17]. It is noteworthy that by evaluating s-ACE in 129 sarcoidosis patients and sarcoidosis suspect patients after genotyping Kruit et al [17] found that 8.5% of these were misclassified as having either normal or elevated s-ACE by application of standard (non-genotype based) reference intervals. Sharma et al [19] evaluated their genotype-based reference intervals on 47 sarcoidosis patients and found 33.5% more patients to have elevated s-ACE than by applying standard reference intervals. These findings indicate that routine genotyping would increase the yield of s-ACE in diagnosing sarcoidosis, though they do not show whether routine genotyping will have a similar impact on the clinical management of these patients. More prospective studies are needed to clarify this.

There is a considerable variation in the results obtained by photometric measurement of s-ACE between analytical methods. This has potential implications for the ability to compare values between laboratories for clinical as well as for scientific purposes. This variation is reduced by using commercial kits, traceable calibrators and by applying external quality control programmes for laboratories [5]. The introduction of routine genotyping increases the clinical significance of small variations in s-ACE, which makes efforts to reduce between-laboratory variation even more important. Whenever I/D genotyping is introduced, genotype-based reference values should be verified with the laboratory kits used for genotyping as well as for measurement of s-ACE activity, preferably at the laboratory performing the analyses, but at least with identical kits in a comparable population group.

Genotyping will certainly incur additional costs to the investigation of sarcoidosis. This has to be taken into account when considering the rationale for performing genotyping. In this context, one approach would be to restrict genotyping to the group of patients in which the impact would expectedly be greatest; at least theoretically, this would be in persons in whom s-ACE is between the upper 97.5 percentile for the genotype II and DD since values in this interval are at the greatest risk of being misclassified as normal or elevated if genotyping is not performed. Prospective studies of the clinical impact of genotyping would help clarify the cost-effectiveness of the analysis and would also help define whether a routine or a selective genotyping approach should be chosen. The use of s-ACE in diagnosing and monitoring of sarcoidosis is challenging itself since it is neither specific, nor sensitive [21], and the clinical justification of the test is a matter of ongoing debate. Since genotyping seems to improve the accuracy of the test, this may very well be cost-effective, though no present studies clearly address this question.

As shown in Table 2, a significant difference in s-ACE between genotypes was also present among a smaller number of verified sarcoidosis cases. It has been demonstrated that the genotype DD is associated with a higher increase in s-ACE than the genotype II [22] in sarcoidosis. The data included in this review did not confirm this trend as the DD/II ratio of s-ACE was lower in sarcoidosis populations than in healthy study populations. However, this assumption is based on two studies only, and it only represents Asian patients. In neither of the two studies were the genotype-based ACE levels

### TABLE 2

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Ethnicity</th>
<th>Assay for s-ACE</th>
<th>Unit</th>
<th>Mean value by genotype</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomita et al [20]</td>
<td>207</td>
<td>Asian</td>
<td>Fujirebio Assay, Tokyo, Japan (colorimetric)</td>
<td>IU/l</td>
<td>II: 21.4, ID: 23.9, DD: 27.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Furuya et al [16]</td>
<td>103</td>
<td>Asian</td>
<td>ACE Color, Fujirebio Inc, Tokyo (colorimetric)</td>
<td>IU/l</td>
<td>II: 18.7, ID: 27.5, DD: 32.7</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

DD = homozygous for deletion; ID = heterozygous; II = homozygous for insertion; s-ACE = serum angiotensin-converting enzyme activity.

### TABLE 3

The frequency of the I/D genotypes between studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Ethnicity</th>
<th>Mean frequency of genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoidosis</td>
<td></td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>Tomita et al [20]</td>
<td>207</td>
<td>Asian</td>
<td>37.2</td>
</tr>
<tr>
<td>Furuya et al [16]</td>
<td>103</td>
<td>Asian</td>
<td>35.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>36.1</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomita et al [20]</td>
<td>314</td>
<td>Asian</td>
<td>43.3</td>
</tr>
<tr>
<td>Furuya et al [16]</td>
<td>341</td>
<td>Asian</td>
<td>46.0</td>
</tr>
<tr>
<td>Kruit et al [17]</td>
<td>200</td>
<td>Caucasian</td>
<td>21.5</td>
</tr>
<tr>
<td>Camos et al [15]</td>
<td>147</td>
<td>Caucasian</td>
<td>20.4</td>
</tr>
<tr>
<td>Sharma et al [19]</td>
<td>146</td>
<td>Caucasian</td>
<td>26.7</td>
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<td>Ruprecht et al [18]</td>
<td>262</td>
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<td>21.0</td>
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<td>Rigat et al [9]</td>
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<td>Biller et al [14]</td>
<td>159</td>
<td>Caucasian</td>
<td>24.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>27.6</td>
</tr>
</tbody>
</table>

DD = homozygous for deletion; ID = heterozygous; II = homozygous for insertion.
stratified for severity of sarcoidosis; therefore, they did
not reveal whether the impact of the I/D genotype on
s-ACE reflects sarcoidosis severity.

We found the I-allele to be more frequent in Asian
than in Caucasian populations, which is in concordance
with prior findings [23]. As there is also a well-known
geographical and ethnic variation in the incidence of sar­
coidosis, it has been hypothesised that the I/D polymor­
phism may play a role in the pathogenesis of sarcoidosis
[16, 24]. Data are conflicting, but most recent studies
generally do not support such a correlation [25, 26]. The
function of the I/D polymorphism, though, is not clearly
understood, but its location in an intronic position sug­
gests a linkage disequilibrium with other transcription­
regulating genes [27].

For genotyping of the I/D polymorphism to be used
routinely, the method has to be stable and reliable.
Previous data have shown that prior PCR techniques
misclassified 4-5% [28, 29] of heterozygote individuals
because the shorter D-allele is amplified more efficiently
that the I-allele. The vast majority of these can be de­
tected by running a confirmatory genotyping on all DD
patients. A recently introduced high-resolution mel­
ting technique provides more robust genotyping results,
with primary HRM results performing at par with pri­
mary and confirmatory RT-PCR results in combination
[12].

Certain limitations apply to this review. Since the
studies were performed over a span of 23 years, the
methods for genotyping and ACE measurement have
changed, which makes direct comparison of studies diffi­
cult. Also, this review only included Asian and Caucasian
subjects. As the I/D prevalence varies between ethnic
groups, the external validity in other ethnic groups might
be limited. All studies showed a significant difference of
s-ACE between genotype groups. We assume that this
effect is due to the I/D genotype playing a major role for
the normal level of s-ACE. This effect could potentially be
enforced by publication bias if studies showing no signifi­
cant difference between groups have not been pub­
dlished. At the single study level, results could be biased
by misclassification of genotypes. In all the identified
studies except one [18], PCR-based genotyping (RT-PCR
or conventional PCR with agarose gel electrophoresis)
was confirmed with a second genotyping.

CONCLUSION

This literature search unequivocally demonstrates that
among Asian and Caucasian persons, the mean s-ACE
activity is significantly higher in individuals with the DD
genotype than in individuals with the II genotype, with
the ID genotype having intermediate values. Few
studies have evaluated the impact of genotyping on the
management of sarcoidosis and though more studies
are clearly needed, the present data suggest that a sig­
nificant amount of sarcoidosis patients are misclassified
because non-genotype-based reference values are ap­
plied. Genotyping will expectedly be of greatest impact
if s-ACE is between the upper 97.5 percentile for the
genotypes II and DD since values in this interval are at
risk of being misclassified, but clinical validation studies
are needed to clarify cost-effectiveness.

Whenever implementing I/D genotyping, genotype­
specific reference levels should always be verified locally
due to great assay variation.

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ACCEPTED: 30 January 2014

CONFLICTS OF INTEREST: none. Disclosure forms provided by the authors
are available with the full text of this article at www.danmedj.dk.

ACKNOWLEDGEMENTS: The study was financed by the Department of Clin­
cial Biochemistry, Aarhus University Hospital, and Department of Pulmonary
Medicine, Aarhus University Hospital. It did not involve any external funding.

FUNDING: The study was financed by the Department of Clinical Biochemi­
try, Aarhus University Hospital, Denmark, and the Department of Pulmonary
Medicine, Aarhus University Hospital, Denmark. No external funding was re­
ceived.

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