Association Between Coronary Heart Disease and the C3F-Gene in Essential Hypertension

BENT Ø. KRISTENSEN, M.D. AND GERT BRUUN PETERSEN, M.D.

SUMMARY The occurrence of the C3F allele was investigated in the following three groups: 69 consecutive referred patients with untreated essential hypertension, including borderline hypertension; 70 patients with established and treated essential hypertension, already attending the same outpatient clinic, and 62 age- and sex-matched normotensive healthy subjects without clinical signs of atherosclerosis or familial predisposition to hypertension.

In the three groups the C3F allele was found in 38.2%, 29% and 20%, respectively. Among the treated hypertensive patients with the C3F gene, 40% had coronary heart disease (CHD) compared to 6.1% among the C3F negative (P < 0.005), and the relative risk of CHD among the treated hypertensive patients with this allele was found to be 10.2 (P < 0.002). The C3F gene was present in 72.7% of the treated patients with CHD. In the untreated patients the occurrence of CHD was low, and no differences between C3F positive and negative patients could be demonstrated. No association of the C3F allele with familial predisposition to hypertension was found.

This study provides further evidence of a positive association of the C3F allele with atherosclerosis, and it is concluded that this allele in a hypertensive patient might accelerate the atherosclerotic process, with subsequent premature development of vascular complications.

GENETIC FACTORS predispose to hypertension and coronary heart disease (CHD). It has been suggested that some of these factors might act via immunological pathways, including the complement system. By using high voltage electrophoresis the C3-protein-component, which has a central place in the complement sequence, has shown a genetic polymorphism comprising a fast and a slow band. This polymorphism is governed by two structural alleles, C3F and C3G. Recently, the C3F allele has been found to be positively associated with atherosclerotic diseases such as angina pectoris, acute myocardial infarction (MI) and claudication.

As hypertension predisposes to CHD, hypertensive patients might be expected to show a higher frequency of the C3F gene with a possible association to vascular complications. In this paper, we present the results from an investigation of the occurrence of the C3F...
gene in: 1) A consecutive series of untreated patients with essential hypertension, including borderline hypertension, 2) a group of patients with established and treated essential hypertension, already attending the outpatient clinic, and 3) a selected group of normotensive individuals without clinical signs of atherosclerosis or familial predisposition to hypertension, and of the same age and sex composition as the untreated hypertensive patients.

Patients and Methods

Of 164 patients with essential hypertension and 80 normotensive control subjects included in a prospective study, it was possible to investigate the C3 phenotypes in 139 patients (85%) and 62 controls (78%), a total of 201 subjects. The untreated patients (consecutive series) comprised 39 males, aged 18–58, mean 38 years, and 30 females, aged 16–61, mean 39 years. Patients with established and treated hypertension comprised 41 males, aged 35–64, mean 53 years, and 29 females, aged 24–60, mean 37 years. The normotensive group (controls) comprised 32 males, aged 18–54, mean 39 years, and 30 females, aged 18–59, mean 41 years. This group was selected from hospital employees and was of the same age and sex composition as the hypertensive patients. All the controls had normal blood pressure (BP) and normal ECG. None had clinical signs of atherosclerotic diseases or familial predisposition to hypertension. Evidence of hypertension among first degree relatives was obtained from a questionnaire. Details concerning selection procedure, diagnostic and exclusion criteria have been published elsewhere.

Blood pressure after 20 minutes at rest was measured in supine and erect positions, using a mercury sphygmomanometer. All recordings were performed by the same specially trained nurse. Erect mean arterial blood pressure (MAP) was used in the following evaluations.

Electrocardiograms were recorded with nine leads, using a multichannel recorder (Elena-Schönander). Ischemia was defined as null or negative T waves in I/II or V5–6, left ventricular hypertrophy as the sum of S in V1 and R6/R6 ≥ 35 mm. Chest x-rays were performed in all but five patients. Heart enlargement was defined as a heart/chest ratio > 0.5. Angina pectoris was defined as exertional chest pains disappearing shortly after rest or nitroglycerin consumption. Congestive heart failure and acute MI were diagnosed according to standard medical procedures.

C3-typing was performed on serum samples using high-voltage starch-gel electrophoresis, according to the method described by Azen et al. Hypertensive patients and control subjects were analyzed simultaneously and the results were read blindly by one of the authors. The clinical data given in this paper refer to status at admission.

Statistics

The chi square test for heterogeneity (with Yate's correction) and Woolf's method on determination of relative risks were used.

Results

Rare C3f variants were found in one control female and one treated male, C3f variants in one control female, and one untreated female. These variants are not included in the calculations.

By counting the number of C3 genes in each of the three groups studied, the C3f gene frequency was found to be 11.8% in the normotensive group, 20.6% in the untreated patients, and 15.9% in the treated patients. Table 1 shows the frequency of C3f positive and C3f negative individuals in the three groups. In the group of untreated patients, the frequency of C3f positive subjects was significantly higher than that in the control group (P < 0.03).

A relation between the C3 gene frequency and age has previously been reported. A valid comparison between the treated hypertensive patients and the normotensive subjects was not possible as the mean age in these two groups was 51 and 40 years, respectively.

Table 2 shows the results from a comparison of clinical data in the untreated patients with and without the presence of C3f. No differences between the two groups could be demonstrated. It should be noted that the average duration of the hypertension was less than two years in both groups and lowest in the C3f positive group (on the average, 14 months). Moreover,

<table>
<thead>
<tr>
<th>TABLE 1. Occurrence of the C3f Gene in Normotensive Healthy Subjects and in Patients with Essential Hypertension. Rare Variants Not Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
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<tr>
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</tr>
<tr>
<td>Normotensive subjects</td>
</tr>
<tr>
<td>Untreated patients</td>
</tr>
<tr>
<td>Treated patients</td>
</tr>
</tbody>
</table>

*Untreated patients vs normotensive (P < 0.03).

<table>
<thead>
<tr>
<th>TABLE 2. Comparison of Clinical Data in C3f Positive and C3f Negative Patients with Untreated Essential Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3f positive</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Sex ratio</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (MAP) mm Hg (mean = SD)*</td>
</tr>
<tr>
<td>Known duration (mean, mo) (range)</td>
</tr>
<tr>
<td>Familial predisposition</td>
</tr>
</tbody>
</table>

*Mean arterial blood pressure (Diastolic BP + 1/3 of pulse pressure).
†Information about the family could not be obtained in one C3f positive and one negative patient.

Abbreviations: MAP = mean arterial blood pressure; CHD = coronary heart diseases.
TABLE 3. Comparison of Clinical Data in C3F Positive and C3F Negative Patients with Treated Essential Hypertension

<table>
<thead>
<tr>
<th></th>
<th>C3F positive</th>
<th>C3F negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>1.22</td>
<td>1.45</td>
</tr>
<tr>
<td>Mean age in years</td>
<td>52</td>
<td>50</td>
</tr>
<tr>
<td>(range)</td>
<td>(30–64)</td>
<td>(24–64)</td>
</tr>
<tr>
<td>MAP mm Hg (mean ± sd)</td>
<td>134 ± 16</td>
<td>133 ± 20</td>
</tr>
<tr>
<td>Known duration (mean, mo)</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>(range)</td>
<td>(2–138)</td>
<td>(1–108)</td>
</tr>
<tr>
<td>Familial predisposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total number of CHD</td>
<td>8 (40%)</td>
<td>3 (6.1%)</td>
</tr>
</tbody>
</table>

*Information about the family could not be obtained in three C3F positive and two C3F negative patients.
†P < 0.005.
Abbreviations: MAP = mean arterial blood pressure; CHD = coronary heart disease.

23% of the C3F positive and 33% of the C3F negative patients had borderline hypertension, defined as MAP between 100 and 115 mm Hg (not shown). In addition, the frequency of familial predisposition to hypertension was not significantly different between the two groups.

Table 3 shows the results from a similar comparison in patients with established and treated essential hypertension. The higher sex (male:female) ratio found in the C3F negative group was not significantly different from that in the C3F positive group. It can be seen that mean age, MAP and duration of the hypertension in the two groups were similar. The frequency of familial predisposition to hypertension was also equal in these two groups of patients. The total frequency of CHD among the C3F positive patients was 40% as opposed to 6.1% among the C3F negative patients (P < 0.005). The frequency of C3F positive patients among those with CHD was 72.7%.

The relative risk of CHD among C3F positive patients was calculated (table 4) using the method described by Woolf. From this table it appears that the relative risk of CHD among patients with established and treated essential hypertension was increased more than in C3F positive compared to C3F negative patients. In the untreated patients the corresponding risk was not increased and a significant difference between the risk in this group and the treated patients was found (P < 0.05).

The frequency of ischemic ECG changes and heart enlargement did not differ significantly between any of the groups investigated. Additionally, no significant associations between the C3F allele and cerebral or renal complications were found.

**Discussion**

In this investigation the frequency of CHD was almost seven times higher among C3F positive than among C3F negative patients with treated essential hypertension, giving a relative risk of 10.2 for CHD in C3F positive patients. In another study of 101 patients less than 65 years of age, atherosclerotic diseases comprised angina pectoris, acute MI and claudication. Sørensen and Dissing found the C3F allele in 46.8% of these patients and the relative risk for atherosclerotic diseases was 1.87 among C3F positive persons. The findings in our study of an occurrence of the C3F allele in about 73% of patients with established and treated essential hypertension and CHD, and the tenfold increased risk of these diseases among patients with this allele, seem to be in agreement with the findings of Sørensen and Dissing. The higher relative risk of CHD among C3F positive patients in the present study might be explained by the coexistence of hypertension, a well-known risk factor of CHD.

The lack of an association between C3F and CHD in the untreated patients might be explained by the rather low frequency of CHD in this series and the differences in age and duration of hypertension between the untreated and treated patients. The inclusion of borderline hypertension did not seem to influence the observed discrepancy, as the frequency of patients with borderline hypertension was almost equal in untreated patients with and without C3F. Surprisingly, C3F was less frequent among the treated patients (29%) than among the untreated patients (38.2%). An investigation by Sørensen and Dissing of the C3-phenotype distribution in relation to age among 2,000 Danish blood donors showed a decrease in the C3F gene frequency after the age of 55 until the ages of 60–65. This decrease was assumed to be due to a negative selection with respect to the C3F gene in the older age groups in the donor population caused by an association between atherosclerotic vascular diseases and the C3F gene. Of our original 84 treated patients included in a prospective study, six died from vascular complications before C3-typing was performed, and since C3F has been found to be more frequent in atherosclerotic diseases, the low frequency of C3F in the treated patients could be an artifact due to selection.

The frequency of C3F positive subjects in the present, highly selected control group was rather low, (20%), but might be expected to be lower than that in a donor population, as some of the donors might have hypertension.

The significance of the complement system for the development of the initial arterial lesions leading to atherosclerosis was suggested by Geertinger and Sørensen. By causing local membrane damage or increasing the permeability of the endothelium and consequent influx of plasma proteins into the arterial wall,
The complement system was thought to contribute to the development of atherosclerosis,10, 14

The positive association of the C3F allele with atherosclerosis was assumed by these authors to be due to an increased activity of the C3-protein-component induced or governed by this allele.

The findings in the present study of a positive association of the C3F allele with CHD in patients with established and treated essential hypertension may therefore be regarded as further support of an association of this allele with atherosclerosis. An association of the C3F gene with familial predisposition to hypertension was not found, and there are no reasons to presume that the C3F allele should play an etiological role in hypertension, but its presence in a hypertensive patient might accelerate the atherosclerotic process with subsequent premature development of vascular diseases.

Acknowledgments

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