Elevated fibrinogen in the healthy male relatives of patients with severe, premature coronary artery disease

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Aims To assess the possible role of certain coagulation factors and associated genetic polymorphisms in families in which coronary disease has occurred prematurely.

Methods and Results One hundred and eighty-five healthy male relatives aged 65 or less were recruited following the identification of 125 patients with confirmed, premature coronary artery disease and compared to a control group of 185 healthy, age-matched volunteers. None of the control subjects had a personal or family history of coronary artery disease. The relatives and controls were similar in terms of conventional coronary artery disease risk factors. Fibrinogen levels were elevated in relatives compared with controls and remained higher after adjustment for significant correlates, 3·0 g. l⁻¹ (2·9–3·1) vs 2·8 g. l⁻¹ (2·8–2·9), P=0·004. Factor VII coagulant activity and von Willebrand factor antigen did not differ between the groups nor were there any differences in genotype frequency for the fibrinogen BÆ-455 G/A polymorphism or the factor VII promoter deletion/insertion and Arg-Gln coding polymorphisms.

Conclusions A significant increase in fibrinogen levels was demonstrated in the healthy, male, first-degree relatives of patients with severe coronary artery disease. Fibrinogen may be of particular importance in subjects who, other than their family history, appear to be at low risk in terms of conventional coronary artery disease risk factors.

Key Words: Family history, coronary artery disease, fibrinogen, factor VII, von Willebrand factor.

Introduction

A family history of premature coronary artery disease is an established risk factor for the development of coronary disease which in several studies has proved to be independent of other biochemical, metabolic and lifestyle risk factors[1–3]. There is persuasive evidence for a genetic component to familial coronary artery disease, in particular, a large study of Swedish monozygotic and dizygotic twins demonstrated increased concordance for coronary artery disease related death in the monozygous group[4]. Concordance persisted, albeit attenuated, even when death of the first twin did not occur until after the age of 65. However, the biological mechanism by which a family history of premature, atherothrombotic coronary artery disease leads to increased risk remains unclear and cannot be fully explained by the clustering of atheromatous risk factors alone[5].

Fibrinogen[6–9], factor VII coagulant activity[6], and von Willebrand factor antigen[10] have been shown in prospective studies to be associated with the development of fatal and non-fatal coronary artery disease events and the recurrence of disease in coronary artery disease patients[11–13]. We aimed to investigate these factors in order to assess the role of the thrombotic component in families in which severe coronary artery disease occurs prematurely.

Methods

One hundred and twenty-five male patients (probands) aged 65 or less at the time of diagnostic coronary angiography and with confirmed two- or three-vessel
coronary artery disease (WHO criteria of ≥ 50% stenosis in a major epicardial vessel) were identified via the surgical revascularization waiting list at the Yorkshire Heart Centre, Leeds. One hundred and eighty-five of their male, first-degree relatives aged 65 or less at the time of recruitment and free from a personal history of coronary artery disease were enrolled in the study. An equal number of male, community control subjects aged 65 or less and without a personal or family history of coronary artery disease or diabetes mellitus were recruited via the Leeds Health Authority Family Health Service register. All subjects were White, North European and gave informed consent according to a protocol approved by the United Leeds Teaching Hospitals (NHS) Trust Research Ethics Committee. The investigation confirms with the principles outlined in the Declaration of Helsinki.

At the time of recruitment all subjects had fasted for a minimum of 10 h overnight. Fifty ml of venous blood was taken from an antecubital vein with a 19-gauge needle without venous stasis with the subject in a supine position. Blood was collected in lithium fluoride for plasma glucose estimation, lithium heparin for lipid fraction analysis, EDTA for DNA extraction and a 10 ml tube containing 1 ml 0.9% citrate (pH 8.8) at room temperature for assay of fibrinogen, factor VII coagulant activity and von Willebrand factor antigen. These citrate samples were centrifuged at 2560 \( \times g \) at room temperature for 20 min and aliquots of 0.5 ml plasma supernatant snap-frozen in liquid nitrogen for storage at \( -40^\circ C \) until assay.

Systolic and diastolic blood pressure measurements were performed manually to the nearest 2 mmHg with the subject in a supine position. Blood was collected in lithium fluoride for plasma glucose estimation, lithium heparin for lipid fraction analysis, EDTA for DNA extraction and a 10 ml tube containing 1 ml 0.9% citrate (pH 8.8) at room temperature for assay of fibrinogen, factor VII coagulant activity and von Willebrand factor antigen. These citrate samples were centrifuged at 2560 \( \times g \) at room temperature for 20 min and aliquots of 0.5 ml plasma supernatant snap-frozen in liquid nitrogen for storage at \( -40^\circ C \) until assay.

Fibrinogen was measured by the Claus method\(^{(15)}\), von Willebrand factor antigen by ELISA (Dako) and factor VII coagulant activity by an ACL 3000 plus (Instrumentation Laboratories) with factor VII-depleted plasma (Sigma) and recombinant thromboplastin (Instrumentation Laboratory). Factor VII coagulant activity was expressed as a percentage of activity given by calibration plasma. Inter-assay and intra-assay coefficients of variation were 3.5% and 2.0%, respectively, for fibrinogen, 4.7% and 2.8% for von Willebrand factor antigen, and 4.3% and 3.2% for factor VII coagulant activity. A glucose oxidase method was used for measurement of plasma glucose and a Hitachi 747 autoanalyzer (Boehringer Mannheim) for estimation of triglyceride and total cholesterol. HDL cholesterol was measured by a Hitachi 717 autoanalyzer (Boehringer Mannheim) after removal of LDL, chylomicrons and VLDL by precipitation with phosphotungstic acid and magnesium chloride. LDL cholesterol was calculated by the Friedewald equation.

In view of evidence that certain fibrinogen and factor VII gene polymorphisms relate to their circulating levels, the genotypes at the fibrinogen B\( ^{\beta} \)-455 G/A polymorphism (classified as G/G, G/A and A/A), the factor VII exon 8 Arg-Gln polymorphism (alleles denoted by the appropriate amino acid abbreviation, Arg or Gln) and the promoter decanucleotide deletion/insertion polymorphism (the more frequent allele, deletion, denoted as ‘D’ and the less frequent insertion allele denoted as ‘I’) were identified using previously described methods\(^{(16,17)}\).

Values for age for the three study groups did not conform to a normal distribution and are presented as medians with 25th and 75th percentiles. Differences in age between groups were assessed by the Kruskal–Wallis test. In order to achieve a normal distribution for fibrinogen, factor VII coagulant activity, von Willebrand factor antigen, plasma fasting glucose and triglyceride their values were log transformed and subsequent data presented as geometric mean with antilog 95% confidence intervals. An Independent Student’s t-test was used to assess differences in continuous, parametric data and a chi-square test for differences in category frequencies. One-way ANOVA was used to identify any relationship between genotype and coagulation factor level. Multiple linear regression analysis was performed for each coagulation factor and the data presented represents the best-fit model as determined by the \( R^2 \) value. Statistical significance was taken as \( P<0.05 \) and all analyses were performed with the Statistics Package for Social Scientists for Windows version 9.0.

**Results**

It can be seen from Table 1 that the relatives and control subject groups were similar in terms of age, smoking habit, anthropometric and blood pressure measurements, fasting glucose and lipid fractions. Medical and drug history data are displayed in Table 2. Ten relatives and five control subjects gave a history of hypertension and were taking anti-hypertensive treatment with standard agents. Exclusion of the 18 relatives of diabetic probands and the 17 subjects receiving cardiovascular medication did not affect the results of the study and these subjects were therefore included in the clinical and biochemical data shown in Table 1. Proband data is included for completeness and as expected the probands were older, with a higher body mass index and waist to hip ratio and lower HDL cholesterol than both the relatives and the control subjects.

**Fibrinogen**

Plasma levels of fibrinogen (Table 1) were significantly higher in relatives (geometric mean and 95% confidence...
The intervals), 3·0 (2·9–3·1) g l\(^{-1}\), compared to control subjects, 2·8 (2·8–2·9) g l\(^{-1}\), with a mean difference of 0·14 g l\(^{-1}\) (P=0·01). On the basis of bivariate correlation coefficients (data not shown), multiple regression analysis was performed using subject status (relative or control), current smoking status (smoker or non-smoker), mean systolic blood pressure, body mass index, waist to hip ratio, HDL and total cholesterol as covariates (Table 3). Of these variables, relatives vs controls, age, HDL cholesterol and current cigarette smoking remained independent predictors of fibrinogen levels. Antilogged coefficient values were used to calculate effect size and this revealed that being a relative, a current cigarette smoker, a 20 year increase in age and a 1 mmol l\(^{-1}\) reduction in HDL cholesterol were each associated with a 1·1 fold increase in fibrinogen levels. Adjusted mean values for fibrinogen were 3·0 (2·9–3·1) g l\(^{-1}\) in relatives and 2·8 (2·8–2·9) g l\(^{-1}\) in controls (P=0·004) and 3·1 (2·9–3·2) g l\(^{-1}\) vs 2·8 (2·8–2·9) g l\(^{-1}\) (P=0·001) in current smokers vs non-smokers.

Separate analysis of the relatives group based on proband history of myocardial infarction (91 relatives with a proband history of previous myocardial infarction vs the 94 relatives without) did not reveal any

### Table 1 Clinical and biochemical data and haemostatic factor levels for the three study groups

<table>
<thead>
<tr>
<th></th>
<th>Relatives (n=185)</th>
<th>Controls (n=185)</th>
<th>Probands (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>36 (19–51)</td>
<td>42 (18–63)</td>
<td>60 (52–68)‡</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>32</td>
<td>24</td>
<td>14†</td>
</tr>
<tr>
<td>BMI, kg m(^{-2})</td>
<td>26·2 (25·6–26·8)</td>
<td>25·6 (25·1–26·2)</td>
<td>28·7 (27·4–29·9)‡</td>
</tr>
<tr>
<td>WHR</td>
<td>0·91 (0·90–0·92)</td>
<td>0·90 (0·89–0·91)</td>
<td>1·03 (1·00–1·06)‡</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>135 (133–137)</td>
<td>132 (130–135)</td>
<td>134 (130–139)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>80 (78–82)</td>
<td>80 (78–81)</td>
<td>77 (74–80)</td>
</tr>
<tr>
<td>Total cholesterol, mmol l(^{-1})</td>
<td>5·2 (5·1–5·4)</td>
<td>5·0 (4·9–5·2)</td>
<td>4·9 (4·6–5·3)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol l(^{-1})</td>
<td>1·3 (1·2–1·3)</td>
<td>1·3 (1·2–1·3)</td>
<td>1·2 (1·0–1·3)*</td>
</tr>
<tr>
<td>LDL cholesterol, mmol l(^{-1})</td>
<td>3·2 (3·0–3·3)</td>
<td>3·1 (2·9–3·2)</td>
<td>3·0 (2·7–3·3)</td>
</tr>
<tr>
<td>Triglyceride, mmol l(^{-1})</td>
<td>1·4 (1·3–1·5)</td>
<td>1·3 (1·2–1·4)</td>
<td>1·6 (1·4–1·9)‡</td>
</tr>
<tr>
<td>Fasting glucose, mmol l(^{-1})</td>
<td>5·1 (5·0–5·3)</td>
<td>4·9 (4·8–5·1)</td>
<td>4·8 (4·5–5·1)</td>
</tr>
<tr>
<td>Fibrinogen, g l(^{-1})</td>
<td>3·0 (2·9–3·1)†</td>
<td>2·8 (2·8–2·9)</td>
<td>3·4 (3·2–3·6)‡</td>
</tr>
<tr>
<td>Factor VIIc, %</td>
<td>102 (98–106)</td>
<td>101 (97–104)</td>
<td>103 (95–111)</td>
</tr>
<tr>
<td>vWF antigen, IU ml(^{-1})</td>
<td>1·03 (0·98–1·08)</td>
<td>1·08 (1·01–1·15)</td>
<td>1·36 (1·22–1·52)‡</td>
</tr>
</tbody>
</table>

Values for age are median (25th and 75th percentiles); body mass index, weight to hip ratio, systolic blood pressure, diastolic blood pressure, HDL, LDL and total cholesterol are mean (95% CI); and for the remainder geometric mean (antilog 95% CI).

*P value <0·05; †P value ≤0·01; ‡P value <0·001 (compared with control group mean value).

### Table 2 Medical and drug history data for the three study groups

<table>
<thead>
<tr>
<th></th>
<th>Relatives (n=185)</th>
<th>Controls (n=185)</th>
<th>Probands (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>—</td>
<td>—</td>
<td>60</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10</td>
<td>5</td>
<td>37</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>—</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>Aspirin</td>
<td>2</td>
<td>2</td>
<td>116</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>4</td>
<td>4</td>
<td>84</td>
</tr>
<tr>
<td>HMGCoA inhibitors</td>
<td>2</td>
<td>—</td>
<td>84</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>2</td>
<td>1</td>
<td>32</td>
</tr>
</tbody>
</table>

### Table 3 Multiple linear regression model incorporating relatives and control subjects with log fibrinogen as the dependent variable

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient (95% CI)</th>
<th>Contribution to variance (%)</th>
<th>Significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final model</td>
<td>21·8</td>
<td></td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Age</td>
<td>0·002 (0·001–0·003)</td>
<td>6·9</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Relatives</td>
<td>0·023 (0·007–0·039)</td>
<td>2·3</td>
<td>&lt;0·005</td>
</tr>
<tr>
<td>Current smokers</td>
<td>0·030 (0·012–0·048)</td>
<td>2·9</td>
<td>&lt;0·005</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0·036 (−0·060 to −0·011)</td>
<td>2·2</td>
<td>&lt;0·005</td>
</tr>
<tr>
<td>BMI</td>
<td>0·002 (0·001–0·004)</td>
<td>0·4</td>
<td>0·2</td>
</tr>
<tr>
<td>WHR</td>
<td>0·085 (0·089–0·258)</td>
<td>0·3</td>
<td>0·3</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0·000 (0·000–0·001)</td>
<td>0·3</td>
<td>0·3</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0·000 (0·000–0·001)</td>
<td>0·2</td>
<td>0·4</td>
</tr>
</tbody>
</table>

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association of fibrinogen levels with proband history of myocardial infarction nor were there any differences in clinical or biochemical data between these groups of relatives.

The fibrinogen Bβ-455 G/A polymorphism genotype frequency did not differ between the relatives and control groups. There was no relationship between genotype and fibrinogen level.

Factor VII and von Willebrand factor

Levels of factor VII coagulant activity and von Willebrand factor antigen were not elevated in relatives when compared with control subjects (Table 1). The genotype frequencies of the factor VII promoter deletion/insertion and Arg-Gln coding polymorphisms were similar for the relatives and control groups and were in tight linkage disequilibrium. The rarer alleles were associated with significantly lower levels of factor VII coagulant activity.

Discussion

In this study we have shown that the healthy, male relatives of patients with severe, premature coronary artery disease have elevated plasma levels of fibrinogen, independent of conventional coronary artery disease risk factors. Previous reports have been inconsistent in their findings concerning fibrinogen levels in families in which coronary events have occurred at a young age but this most likely reflects significant differences in study design and methodology. The present study has two principle strengths which reinforce the validity of its overall findings: the recruitment of well characterized, highly comparable relatives and community control subjects and the unequivocal nature of the family history of severe, premature coronary artery disease. In addition, the high degree of similarity between the relatives and controls in terms of lifestyle, clinical and biochemical risk markers suggests that the fibrinogen results are not simply due to the confounding effects of such factors.

Recruitment bias cannot be ignored in studies of case/control design. Of the first-degree relatives who met the study criteria less than 10% were unwilling to participate in the study, most often for geographical reasons. The relatives and control subjects were also recruited concurrently from all parts of the Yorkshire region in approximately equal numbers over a 12 month period, gave negative responses to the Rose Angina Questionnaire and did not have any features of myocardial ischaemia on their resting ECG. These factors would all mitigate against significant recruitment bias.

Subclinical coronary artery disease cannot be excluded in the relatives (or, indeed, the control subjects) and therefore increased fibrinogen may well reflect the inflammatory component of underlying atherosclerotic plaques. Elevated C-reactive protein levels have also been found in the offspring of patients with premature myocardial infarction and since both fibrinogen and C-reactive protein predict coronary artery disease events in healthy subjects, there is persuasive evidence for an association between inflammation and clinical coronary disease. In addition to its role as an acute phase protein, increased fibrinogen is known to enhance platelet aggregation, increase smooth muscle cell proliferation and migration and affect the structure and function of cross-linked fibrin. Fibrinogen may therefore have a more direct role in the development of atherothrombotic coronary artery disease.

Genetic variation at the fibrinogen locus has been reported to influence circulating fibrinogen levels and studies investigating the heritability of fibrinogen support a modest genetic role. The rarer allele of the Bβ-455 G/A polymorphism (which is in linkage disequilibrium with several other β chain gene polymorphisms) has been associated with elevated fibrinogen levels but no such association was found in this study. However, findings in other studies have been inconsistent and a link between these polymorphisms and coronary artery disease has not been established.

Factor VII coagulant activity was not elevated in either relatives or probands when compared with control subjects in this study. Much of the data concerning factor VII coagulant activity and coronary artery disease has been inconsistent with both positive and negative prospective and case-control studies reported. Increased levels may be specifically related to fatal coronary events and in the ECTIM study, the finding of elevated factor VII coagulant activity in the control group may have been due to the premature death (and therefore lack of representation) of those patients with the highest levels of factor VII coagulant activity. However, subtle but important differences in the factor VII coagulant activity assays used by the various groups have been identified and the precise relationship between factor VII coagulant activity and coronary artery disease is not firmly established.

von Willebrand factor antigen levels were elevated in probands compared with control subjects but not in relatives. The association of elevated von Willebrand factor antigen levels with coronary artery disease has been observed in case-control studies although some negative studies have also been reported. Circulating plasma von Willebrand factor antigen levels are known to be influenced by metabolic and environmental factors including age, lipids and the ABO blood system as well as recent evidence, albeit inconclusive, that certain promoter polymorphisms may have some effect on circulating von Willebrand factor antigen levels. In this study, we did not adjust von Willebrand factor antigen levels for the ABO blood group, however, it seems unlikely given the size of our study population that blood group distribution would be significantly skewed. In contrast to the high von Willebrand factor antigen levels observed in probands, we would not
expect to find such an increase in plasma levels of von Willebrand factor antigen in the relatives group and, therefore, our negative findings in relatives may simply reflect the degree to which a multitude of metabolic and environmental factors contribute to the variance of von Willebrand factor antigen.

In this study, we have demonstrated a small but statistically significant increase in fibrinogen levels in the male, first-degree relatives of patients with severe, premature coronary artery disease. The robust design and methodology of the present study suggest that this finding is unlikely to be the result of recruitment bias or confounding factors. However, whether the origin of this increase in fibrinogen is due to the inflammatory component of pre-clinical atherosclerosis or the shared genetic and environmental factors within these families remains speculative. Additional, large prospective studies are required in order to establish the potential role of fibrinogen in the risk stratification of individuals who have a family history of premature coronary disease. Fibrinogen may be of particular importance in those individuals who appear to be at low risk in terms of conventional coronary artery disease risk factors.

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