Mechanisms of disease

Activation markers of coagulation and fibrinolysis in twins: heritability of the prethrombotic state

Robert A S Ariëns, Marlies de Lange, Harold Snieder, May Boothby, Tim D Spector, Peter J Grant

Summary

Background Activation markers of coagulation and fibrinolysis are increased in individuals at risk of coronary-artery disease and other thrombotic disorders—a condition defined as the prethrombotic state. We aimed to find out the extent to which the prethrombotic state is determined by genetic factors.

Methods We analysed concentrations of prothrombin, prothrombin fragment 1+2, thrombin–antithrombin complex, crosslinked fibrin degradation product D-dimer, and thrombin-activatable fibrinolysis inhibitor by ELISA in 118 monozygotic and 112 dizygotic unselected female twins aged 21–73 years from the St Thomas’ UK Adult Twin Registry. We used quantitative genetic-model fitting to estimate heritability.

Findings We found significant heritabilities in concentrations of the activation markers in plasma. Genetic factors contributed 45, 40, and 65% of the variation in concentrations of fragment 1+2, thrombin–antithrombin complex, and D-dimer, respectively. Age was important only in fragment 1+2 concentrations, in which it accounted for 12% of the variation. The remaining variation could be attributed to unique environmental factors. Variation in concentrations of precursor prothrombin in plasma was determined by 57% heritability, and that of zymogen thrombin-activatable fibrinolysis inhibitor showed a very strong genetic component (82%).

Interpretation The activation mechanisms of the coagulation and fibrinolytic systems, and therefore the prethrombotic state, are controlled to a substantial degree by genetic factors. Genes influencing activation of haemostasis are likely to be an important component of the overall thrombotic tendency in the general population.

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Introduction

Coagulation is triggered by the exposure of blood to TISSUE FACTOR, leading to rapid activation of factors VII, IX, and X. Activated factor X cleaves PROTHROMBIN to produce thrombin and FRAGMENT 1+2 (F1+2)—an amino-terminal activation product with a molecular weight of 35–5 kDa.1,2 In the circulation, some of the thrombin generated by these reactions exists in a complex with its main inhibitor, antithrombin.3 Free thrombin converts fibrinogen into fibrin, and thrombin-activated factor XIII cross-links fibrin to increase stability of the clot. Cross-linked fibrin is lysed by plasmin, which regulates clot-formation in space and time. Activation of fibrinolysis can be measured with D-DIMER, which arises from proteolysis of cross-linked fibrin γ chains.3,4 Fibrinolysis rates are inhibited by THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR (TAFI). On activation by the thrombin–thrombomodulin complex, TAFI reduces the affinity of plasminogen for its substrate fibrin by cleaving the C-terminal lysine residues to which plasminogen binds.3,5 Additionally, activated TAFI inhibits plasmin generation catalysed by a complex of tissue plasminogen activator and fibrin degradation product (DD)E.3

Although atherothrombotic and venous thromboembolic disorders have differing patterns of genetic and environmental risk markers, both have in common the formation of fibrin as the endpoint of activation of coagulation. Several studies, including our own, have investigated the HERITABILITY of coagulation precursor proteins, measured as the antigen, in population-based cohorts of twins and families.4–15 Although varying degrees of heritability have been described, whether these observations translate directly into an inherited risk for thrombosis in the normal population is unclear. Enzymatic activation products of the coagulation and fibrinolysis cascades such as F1+2, THROMBIN-ANTITHROMBIN COMPLEX (TAT), and D-dimer are not coded for by specific single genes, and their concentrations reflect overall activation of the coagulation and fibrinolytic systems. Identification of a heritable component in the variation of activation markers would be the first evidence that a genetically determined PRETHROMBOTIC STATE exists constitutively in the otherwise normal population.

We aimed to investigate the hypothesis that activation of the coagulation and fibrinolytic systems has a genetic component. We did this by investigating the heritability of markers of activation of these pathways (F1+2, TAT, and D-dimer) in addition to prothrombin and TAFI in a population of healthy monozygotic and dizygotic female twins.

Methods

Twins All participants were healthy, white, female twin pairs drawn from the St Thomas’ UK Adult Twin Registry. Twins from the registry are unselected volunteers ascertained from the general population through national...
media campaigns in the UK. Participating twins were unaware of the specific hypotheses tested. The study was approved by the St Thomas’ Hospital Research Ethics Committee, and informed consent was obtained from each individual. We used a standardised nurse-administered questionnaire to gather information on medication and demographic variables. Zygosity was ascertained by questionnaire to gather information on medication and individual. We used a standardised nurse-administered approved by the St Thomas’ Hospital Research Ethics unaware of the specific hypotheses tested. The study was taken within 5 min. We kept the samples at room 1000 h after an overnight fast. Blood from the co-twin was parts blood to one part citrate—between 0800 h and anticoagulant (Becton Dickinson, Oxford, UK)—nine tubes containing 0·13 mol/L trisodium citrate as Procedures

CONTACT ACTIVATION
Reciprocal activation of factor XII and kallikrein activates the coagulation, fibrinolysis, complement, and kinin pathways.

D-DIMER
A 182-6 kDa fragment cleaved from cross-linked fibrin by plasmin.

DNA FINGERPRINTING
Investigation of genetic identity by comparison of DNA on electro-phoresis gels after fragmentation of the samples with restriction enzymes.

HEIRATIBILITY
The proportion of variation of a given parameter that can be attributed to additive genetic factors.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)
A nuclear hormone receptor that acts as a transcription factor on binding of its ligands including prostaglandins, leukotrienes, and thiazolidinediones.

PRETHROMBOTIC STATE
A condition defined by increased concentrations of activation markers such as F1+2, TAT, and D-dimer above the normal range.

PROTHROMBIN (FACTOR II)
Precursor form of thrombin—a pivotal enzyme in the activation of haemostasis.

PROTHROMBIN FRAGMENT 1+2 (F1+2)
A large fragment of 35-5 kDa which is cleaved from prothrombin by activated factor X during conversion of prothrombin to thrombin.

THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR (TAFI)
A 60 kDa protein of the carboxypeptidase family that, on activation by the thrombin–thrombomodulin complex, inhibits fibrinolysis by interfering with the binding of plasmin to fibrin and tissue plasminogen activator to fibrin degradation product (DD).

THROMBIN–ANTITHROMBIN COMPLEX (TAT)
Some of the thrombin formed during activation of coagulation circulates in complex with its natural inhibitor, antithrombin.

TISSUE FACTOR (TF)
A transmembrane protein of 53 kDa, normally expressed by extravascular cells only, that through binding of factor VII activates the coagulation pathway.

Statistical analysis
We transformed concentrations of prothrombin, F1+2, and D-dimer by natural logarithm, and TAT by calculating the reciprocal value, to obtain normal distributions; TAFI was normally distributed. Abnormally high concentrations of F1+2 and TAT sometimes occur because of activation of the blood sample during sampling, handling, or storage procedures. This artefact represents in-vitro activation and not true in-vivo concentrations. Elimination of twin pairs in whom at least one of the pair was an outlier (>4 SD above the mean) led to the exclusion of four pairs in the case of F1+2, and three pairs for TAT.

The potential confounding effects of body-mass index, cigarette smoking, menopausal state, and use of oral contraceptive pills and hormone replacement therapy on variation of prothrombin, F1+2, TAT, D-dimer, and TAFI was analysed by generalised estimating equation, which takes the non-independence of the twin data into account, and by regression analysis with cluster and robust options.

We used genetic-model fitting techniques to obtain estimates of the genetic and environmental factors. These techniques make optimum use of the information available in the twin–co-twin covariance structure. Model fitting is based on the comparison of the observed and expected variance-covariance matrices in monozygotic and dizygotic twin pairs. It allows separation of the observed phenotypic variance into additive genetic variance (A), dominance genetic variance (D), common (or shared) environmental variance (C), and unique environmental variance (E), which also contains measurement error, so that the total (or phenotypic) variance (Vp) equals A+D+C+E. Division of each of these components by the total variance yields the different standardised components of variance, for example the heritability (h²) which can be defined as the proportion of overall phenotypic variation that can be explained by additive genetic factors.

Model fitting was done according to the rules of parsimony, to produce a model with the least possible number of parameters to account for the observed variation in plasma concentrations. By incorporating age into the
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Results
We grouped the individuals according to zygosity. The mean age of the group of monozygotic twins was not different from that of the dizygotic twins, nor was there any other significant difference in the general characteristics of the monozygotic and dizygotic twins (table 1). None of the transformed markers of activation (log F1+2, log D-dimer, and reciprocal TAT), log prothrombin, or TAFI were significantly different between the monozygotic and dizygotic twins (table 1). Body-mass index were significantly different between the monozygotic and dizygotic twins (table 1). None of the quantitative genetic modelling was done with Mx software (Virginia Commonwealth University, Richmond, VA, USA).

Table 1: Characteristics of monozygotic and dizygotic twins

<table>
<thead>
<tr>
<th></th>
<th>Monozygotic twins</th>
<th>Dizygotic twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>53.1 (11.5)</td>
<td>52.4 (8.3)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>25.1 (3.9)</td>
<td>26.0 (4.9)</td>
</tr>
<tr>
<td>Number of monogausals</td>
<td>72 (61%)</td>
<td>60 (54%)</td>
</tr>
<tr>
<td>Number on hormone replacement</td>
<td>27 (23%)</td>
<td>39 (35%)</td>
</tr>
<tr>
<td>Number on oral contraceptives</td>
<td>9 (8%)</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Number of smokers</td>
<td>17 (14%)</td>
<td>25 (22%)</td>
</tr>
</tbody>
</table>

Table 2: Twin-pair correlation coefficients (r) of the activation markers

<table>
<thead>
<tr>
<th></th>
<th>Monozygotic twins</th>
<th>Dizygotic twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>0.48</td>
<td>0.38</td>
</tr>
<tr>
<td>F1+2</td>
<td>0.62</td>
<td>0.42</td>
</tr>
<tr>
<td>TAT</td>
<td>0.39</td>
<td>0.25</td>
</tr>
<tr>
<td>D-dimer</td>
<td>0.71</td>
<td>0.22</td>
</tr>
<tr>
<td>TAFI</td>
<td>0.84</td>
<td>0.42</td>
</tr>
</tbody>
</table>

n=number of twin pairs. F1+2=prothrombin fragment 1+2; TAT=thrombin-antithrombin complex; TAFI=thrombin-activatable fibrinolysis inhibitor.

Discussion
Concentrations of F1+2, TAT, and D-dimer in plasma, which increase on activation of coagulation and fibrinolysis, are a measure of thrombotic tendency. Under normal conditions, the coagulation and fibrinolytic pathways sustain low-grade activation, as measured by circulating markers of activation in healthy individuals. In thrombotic conditions such as coronary-artery disease and stroke, concentrations of these markers increase significantly.16,17 Heritability of circulating concentrations of clotting factor zymogens have been reported previously,16,17 but the relation between these concentrations and disease is indirect. In the present study, we determined the extent to which individual differences in molecular activation products of the coagulation and fibrinolysis systems, F1+2, TAT, and D-dimer, are heritable in a healthy population. The results show a striking contribution of genetic factors to the variation in concentrations of these activation markers, ranging from 40 to 65% of the total phenotypic variance, providing evidence for a significant genetic basis to the prethrombotic state in otherwise healthy individuals. We also found a high heritability of 57% for prothrombin and 82% for TAFI.

Several studies have reported significant genetic control on the variation of precursors in coagulation and fibrinolysis,18,19 ranging from 24% for plasminogen to 82% for TAFI and factor XIII activity (figure). In the present study, variation in molecular markers of activation of the enzymatic pathways also had a high degree of heritability,
similar to that of the zymogen precursors. The heritability estimates of two molecular products of thrombin generation, F1+2 and TAT, were almost as high as that of prothrombin. Variation in concentrations of D-dimer and TAFI in plasma showed a heritable component of 66% and 82%, respectively, indicating the presence of a high degree of genetic control over the complex processes of fibrin formation and fibrinolysis.

Our findings raise challenging questions about regulation of haemostatic processes and risk of thrombosis. Heritable variation of haemostasis and fibrinolysis proteins encoded by single genes is comprehensible without too much difficulty. Heritability of activation markers in an enzymatic cascade is likely to be more complex, since these markers are not the product of a single gene. The enzymatic cascade is started by tissue factor, which is expressed on the membrane of extravascular cells to which blood is exposed on damage to the vasculature. An alternative pathway involves the contact activation system, which, in vitro, seems to have additional effects on regulation of the fibrinolytic system. Activation of these pathways leads to thrombin generation and increased concentrations of the activation markers measured in the current study. The existence of basal concentrations of activation markers has led to the view that the coagulation and fibrinolytic systems are primed to respond to thrombotic stimuli, but are normally in equilibrium to prevent either excessive bleeding or thrombosis. Heritability of activation markers is unlikely to be the sum of the heritabilities of the coagulation proteins, since this would not explain mechanisms of activation of the enzymatic cascade.

Our unpublished data from further studies in the twin cohort and in a Leeds-based family study both show a substantial degree of pleiotropy between insulin resistance and concentrations of coagulation proteins. This finding indicates that common genetic and environmental factors underpin the association between otherwise unrelated risk markers. If this is the case, common regulatory elements in the cell such as transcriptional activators and mRNA binding proteins might be candidates for regulation of activation of haemostasis through responses to vascular damage. A clinical example in support of this is shown by the PEROXSOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) family of transcriptional activators, of which PPARy is best known, in relation to the development of thiazolidinediones used in the management of type 2 diabetes. Thiazolidinediones are PPARy activators and their use is associated with a reduction in circulating insulin and glucose, effects on adipocyte differentiation, and reduction in circulating concentrations of the fibrinolytic inhibitor, plasminogen activator inhibitor 1. Perhaps more importantly, there is evidence that this family of drugs reduces inflammatory responses, which are thought to cause vascular damage and to activate coagulation. There might be several transcriptional activators with similar effects to the PPARy family, which would provide an overarching regulation of coagulation processes and account for the heritability of activation markers described in this study. If these hypotheses are proven to be correct, further study of transcriptional regulation of coagulation processes holds out the prospect of the development of novel anticoagulant therapies based on targeting of transcriptional activators in a manner already successfully applied to the management of type 2 diabetes.

There are major implications of a strong genetic influence on the prethrombotic state for risk assessment of myocardial infarction, venous thrombosis, and stroke. The importance of a family history of cardiovascular disease has been well established. The finding of significant heritability for the variation in circulating F1+2, TAT, and D-dimer concentrations suggests that there is an inherited variability in the prethrombotic state in otherwise healthy individuals, and transcriptional activators could possibly have a role in this variation. Further elucidation of the molecular mechanisms involved in the inheritance of activation of haemostasis and fibrinolysis could be important for our understanding of the role of activation of the two pathways in determining risk of thrombosis, and might indicate new therapeutic targets for the treatment of the disease.

Contributors
Robert Arians and May Boothby assessed quality control and did the biochemical assays; Marlies de Lange and Harold Sniieder did the statistical analysis and model fitting procedure; Tim Spector and Peter Grant contributed to the design of the study; and all investigators contributed to the writing and review of the paper.

Conflict of interest statement
None declared.

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