

The genetics of haemostasis: a twin study

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Summary

Background The concentrations of fibrinogen, factor VII and VIII, von Willebrand factor, plasminogen activator inhibitor-1 (PAI-1), and tissue plasminogen activator have been associated with coronary-heart disease. In addition, polymorphisms in the genes coding for fibrinogen, factor VII, PAI-1, and factor XIII have been reported to affect both protein concentrations and cardiovascular disease risk.

Methods We did a classic twin study to assess heritabilities of these haemostatic factors. We enrolled 1002 female twins; 149 pairs of monozygotic and 352 pairs of dizygotic twins. 89 monozygotic and 196 dizygotic twin pairs were analysed for factor VII.

Findings Quantitative genetic model fitting showed that genetic factors contributed to about 41–75% of the variation in concentrations of fibrinogen, factor VII, factor VIII, PAI-1, tissue plasminogen activator, factor XIII A-subunit and B-subunit, and von Willebrand factor. Factor XIII activity showed higher (82%) and factor XIIIa lower (38%) heritability.

Interpretation We have shown that genetic factors have a major effect on plasma concentrations of haemostatic proteins. Our results stress the importance of research into the genetic regulation of proteins involved in haemostasis and atherothrombotic disorders, including myocardial infarction and stroke.

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Introduction

The risk of coronary-heart disease (CHD) is partly determined by a family history of this disease.¹ This familial risk could be due to shared genetic influences or shared environmental factors, or, as is most likely, a combination of the two. Only twin or adoption studies are able to discriminate between these sources of familial resemblance. Studies from the Scandinavian twin registries reported an increased concordance for risk of death from CHD in monozygotic twins compared with dizygotic twins, suggesting that sharing genes is more important than sharing family environment in determining mortality from cardiovascular disease.²

These genetic influences on CHD are most likely to be mediated through genetic influences on its risk factors. Many traditional risk factors such as hypertension, dislipidaemia, obesity, and even behaviourally-related risk factors like smoking, physical exercise, and diet are known to be affected by genetic factors. However, even after correction for these traditional risk factors, there is still a large genetic influence on mortality of CHD.²

Prospective and case-control studies have identified abnormalities in both coagulation and fibrinolysis as important risk factors in development of myocardial infarction. The importance of intracoronary thrombosis in myocardial infarction is well established and increased circulating concentrations of fibrinogen, factor VII, tissue plasminogen activator, and plasminogen activator-inhibitor-1 (PAI-1) predict the occurrence of myocardial infarction in middle-aged healthy people,^{3–6} in those known to have coronary atheroma, and in young hyperlipidaemic people with previous myocardial infarction (PAI-1).^{3–7}

Only a few studies have reported the extent of genetic effects on individual haemostatic factors, showing moderate familial clustering for fibrinogen, PAI-1, factor VII and VIII, and von Willebrand factor.^{8–13} In this study, we used a large sample of female twin pairs to apply quantitative genetic model fitting to estimate and contrast genetic and environmental effects on the proteins of coagulation and fibrinolysis.

Methods

Participants

A total of 501 female Caucasian twin pairs (149 monozygotic and 352 dizygotic) aged 18–79 years from the St Thomas' UK Adult Twin Registry participated in the study. For the analysis of factor VII, we took a subsequent sample of 285 female Caucasian twin pairs (89 monozygotic and 196 dizygotic twin pairs) aged 20–76 years from the same registry. Twins from the registry were ascertained from the general population through national media campaigns in the UK. Participating twins were unaware of the specific hypotheses tested and informed consent was obtained from

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all. The study was approved by the St Thomas' Hospital research ethics committee. We established zygosity by standardised questionnaire, which was confirmed by DNA fingerprinting. Information on medication use, demographic variables, and fasting time was obtained by standardised nurse-administered questionnaire.

Five twins (one dizygotic twin pair, three dizygotic twins) were on oral anticoagulants and were excluded from the analyses. Four dizygotic twin pairs and two monozygotic twins were excluded because plasma samples were lost during processing, which resulted in 147 complete monozygotic and 344 complete dizygotic twin pairs for analysis. In the sample for factor VII, one monozygotic and one dizygotic twin were on oral anticoagulants and were excluded from analysis. One dizygotic twin was not analysed because of loss of plasma during processing, resulting in 282 complete twin pairs (88 monozygotic and 194 dizygotic twin pairs). The singletons were kept in the analysis and contributed to the estimates of variance.

Blood sampling and biochemical assays

We took venous blood samples between 8 and 10 am after overnight fasting. Within 5 min blood was taken from the co-twin into 0.13 trisodium citrate vacutainers (Becton Dickinson, Oxford, UK) and kept on ice for tests of fibrinolysis (PAI-1 and tissue plasminogen activator), and at room temperature for tests of coagulation (factor VII, VIII, and XIII, von Willebrand factor, factor XIIa, and fibrinogen). Within 1 h of collection, samples were centrifuged at 2560 g for 20 min to obtain platelet-poor plasma, frozen in aliquots in liquid nitrogen, and stored at -40°C until analysis. We did biochemical assays within 6 months of sample collection.

We selected coagulation or fibrinolytic factors that have been previously associated with risk for atherothrombotic disorders. We used immunosorbent assays to measure amount of circulating proteins, ELISA (Immuno, Vienna, Austria) for factor VIII concentrations; an in-house sandwich ELISA¹⁴ for von Willebrand factor; and ELISAs from Biopool (Umeå, Sweden) for PAI-1 and tissue plasminogen activator. For measurement of fibrinogen and factor VII, however, functional clotting assays were chosen similar to those used in the studies relating these factors to vascular risk.^{3,4} Fibrinogen concentrations were measured according to Clauss,¹⁵ and factor VII with a prothrombin-time based clotting assay with factor VII-deficient plasma on an ACL300 research coagulometer (Instrumentation Laboratory, Milan, Italy). For factor XII, we used an ELISA (Axis-Shield, Dundee, Scotland) specific for the activated form of this factor, which has been related to the risk of coronary-artery disease.¹⁶ A common polymorphism in factor XIII (Val34Leu) is currently under investigation as a risk factor for vascular disease.¹⁷ Therefore, we used immunosorbent assays to measure each factor XIII subunit, and did an activity assay sensitive to Val34Leu. We analysed factor XIII A-subunit and B-subunit with in-house sandwich ELISAs and factor XIII activity with a 5(biotinamido)pentylamine incorporation assay.^{18,19}

Quantitative genetic model fitting of twin data

Genetic model fitting techniques were used to obtain estimates of the genetic and environmental factors. These techniques make optimum use of the information available in the twin and co-twin covariance structure.²⁰ Model fitting is based on the comparison of the observed and expected variance-covariance matrices. The reported phenotypes are assumed to be linear functions of the underlying additive genetic variance (A), dominance

genetic variance (D), common environmental variance (C), and unique environmental variance (E), which also contains measurement error, so that the total (or phenotypic) variance (V_p) equals: $A+D+C+E$. Division of each of these components by the total variance gives the different standardised components of variance, for example the heritability (h^2) which can be defined as the proportion of overall phenotypic variation that can be explained by additive genetic factors.²⁰

Statistical analysis

We log transformed all haemostatic factors before analysis to obtain normal distributions, avoiding bias in the maximum likelihood model fitting results. The effect of potential confounding variables was accommodated in the model by incorporating the effect of age, date of blood sampling (ie, seasonal variation), fasting status, and use of aspirin as a linear regression on the trait value. Fasting status was incorporated into the model because a minority of the twins were not fasting from midnight, and previous studies have shown a potential effect on certain haemostatic factors. Fasting status was coded as either yes (fasting ≥ 8 h) or no (fasting < 8 h) and date of blood sampling as number of days from the start of the study. The significance of these variables on haemostatic factors was tested by investigating whether their regression coefficients could be set to zero without a large reduction in fit of the full model of additive genetic, common environmental, and unique environmental variance.

Model fitting procedure

Models were fitted to the raw data by the normal theory maximum likelihood.²⁰ The significance of A, C, and D can be tested by removing them sequentially in specific submodels and comparison with the full model. Standard likelihood-ratio tests between models were used to assess the importance of each variance component (A, C, or D) on the fit of the model, allowing for the use of twin (non-independent pairs) data.²⁰ This eventually leads to a model in which the pattern of variances and covariance is explained by as few variables as possible. Estimates of the quantitative genetic variables and their 95% CIs were obtained from the best-fitting model. All quantitative genetic model-fitting was done with Mx, a computer program specifically designed for the analysis of twin and family data.

	Monozygotic	Dizygotic
Age (years)	51.54 (13.25)	49.20 (12.29)
Height (m)	1.61 (0.06)	1.62 (0.06)
Weight (kg)	65.77 (11.92)	66.60 (12.31)
Body mass index (kg/m ²)	25.25 (4.31)	25.36 (4.61)
Postmenopausal (%)	60.3	49.3
Current aspirin use (%)	5.8	3.4
Current hormone replacement therapy use (%)	23.7	20.3
Current oral contraceptive pill use (%)	9.9	8.9
Current smokers (%)	17.2	22.7
Fibrinogen (g/L)	3.22 (0.75)	3.02 (0.73)
Factor VII (%)	104.56 (20.74)	103.55 (23.78)
Factor VIII (IU/mL)	0.89 (0.32)	0.87 (0.31)
PAI-1 (ng/mL)	9.33 (8.07)	11.02 (10.62)
Tissue plasminogen activator (ng/mL)	6.66 (3.42)	6.88 (3.78)
von Willebrand factor (IU/mL)	1.15 (0.40)	1.16 (0.40)
Factor XIIa (ng/mL)	2.69 (1.01)	2.60 (0.98)
Factor XIII activity (%)*	111.02 (31.64)	107.62 (33.41)
Factor XIII A-subunit ($\mu\text{g/mL}$)†	1.15 (0.33)	1.05 (0.27)
Factor XIII B-subunit ($\mu\text{g/mL}$)†	1.14 (0.38)	0.99 (0.27)

Values are mean (SD), unless stated otherwise.

*Concentrations expressed as % of pooled normal plasma.

†Concentrations expressed as units/mL of pooled normal plasma. PAI-1=plasminogen activator inhibitor-1.

Table 1: General characteristics and concentrations of proteins in haemostasis and fibrinolysis in twin pairs

	Monozygotic		Dizygotic	
	Number of complete pairs	Intraclass correlations	Number of complete pairs	Intraclass correlations
Fibrinogen	145	0.51	336	0.34
Factor VII	88	0.55	194	0.42
Factor VIII	145	0.66	338	0.40
PAI-1	141	0.55	339	0.37
Tissue plasminogen activator	137	0.65	324	0.47
von Willebrand factor	145	0.79	340	0.41
Factor XIIIa	144	0.64	339	0.42
Factor XIII activity	145	0.80	341	0.39
Factor XIII A-subunit	141	0.78	334	0.37
Factor XIII B-subunit	141	0.79	334	0.54

PAI-1=plasminogen activator inhibitor-1.

Table 2: Number of complete pairs and intraclass correlations for monozygotic and dizygotic twin pairs

Results

Table 1 shows the general characteristics and mean concentrations of haemostatic factors for monozygotic and dizygotic twin pairs. Since dizygotic twins were on average 2 years younger than the monozygotic twins, there was a lower number of postmenopausal dizygotic twins. Other characteristics were similar in monozygotic and dizygotic twins. Concentrations of the (log transformed) haemostatic factors were not significantly different, except for fibrinogen, factor XIII A-subunit and B-subunit, which were slightly higher in the monozygotic twins. The differences in age, fibrinogen, factor XIII A-subunit and B-subunit were accounted for in model fitting. In the sample for factor VII, no difference in age was recorded between monozygotic and dizygotic twins, nor in concentrations of factor VII or any other characteristics. Table 2 presents the intraclass correlation of the monozygotic and dizygotic twins. A higher monozygotic than dizygotic twin correlation is seen for all factors, which suggests that genetic factors affect concentrations of the haemostatic factors.

We analysed the data with quantitative model-fitting to estimate the genetic and environmental variance components. Table 3 shows the standardised variance components for the best-fitting model and 95% CIs for the measured haemostatic factors. For most factors, the data could best be explained by a model with an additive genetic and a unique environmental component. The additive genetic component explained substantial proportions of the total variance, ranging from 44% for fibrinogen to 82% for factor XIII activity (table 3). For factor XIIIa and XIII B-subunit, a small common environmental effect was detected with additive genetic effects. No significant dominance component was recorded for any of the haemostatic factors.

The effect of age on the haemostatic factors was significant for all factors and explained 1.5% (for PAI-1)

Haemostatic factor	A	C	E
Fibrinogen	0.44 (0.32–0.54)	..	0.56 (0.46–0.68)
Factor VII	0.63 (0.50–0.72)	..	0.37 (0.28–0.50)
Factor VIII	0.61 (0.52–0.69)	..	0.39 (0.32–0.48)
PAI-1	0.60 (0.51–0.68)	..	0.40 (0.32–0.49)
Tissue plasminogen activator	0.62 (0.53–0.69)	..	0.38 (0.31–0.47)
von Willebrand factor	0.75 (0.68–0.80)	..	0.25 (0.20–0.32)
Factor XIIIa	0.38 (0.13–0.62)	0.23 (0.03–0.41)	0.39 (0.31–0.49)
Factor XIII activity	0.82 (0.76–0.86)	..	0.18 (0.14–0.24)
Factor XIII A-subunit	0.64 (0.54–0.71)	..	0.36 (0.29–0.46)
Factor XIII B-subunit	0.41 (0.19–0.62)	0.26 (0.08–0.42)	0.33 (0.26–0.43)

A=additive genetic variance, C=common environmental variance, E=unique environmental variance.

Table 3: Estimates and 95% CI of genetic and environmental variance

to 14.4% (for tissue plasminogen activator) of the variance as modelled in the linear regression. There was some seasonal variation in fibrinogen, factor VIII, and XIII A and B-subunit, but this was accounted for in the model fitting. Neither fasting nor the use of aspirin had a substantial effect on any of the haemostatic factors.

Discussion

We have comprehensively quantified the genetic and environmental effects on the multiple key proteins of haemostasis. High heritabilities were reported for most haemostatic factors, and the highest heritability was estimated for factor XIII activity. Age had a significant effect on all factors, with concentrations increasing with age.

Our heritability estimate for fibrinogen agrees with previous family studies; 51% heritability extrapolated from the family study of Hamsten and colleagues,⁸ and 20% to 48% from the Kibbutzim Family Study.⁹ Fibrinogen consists of three chains (A α , B β , and γ), encoded by three genes in close proximity on the long arm of chromosome four. Several polymorphic sites have been identified on the B β chain which seem to make minor contributions (about 5%) to variation in concentrations,¹⁷ suggesting that there is still a substantial genetic effect on fibrinogen concentrations which is yet to be identified. Concentrations of fibrinogen vary widely in populations, perhaps partly because of the acute phase response, and major genetic effects which reside in the factors could regulate this response.

Raised concentrations of PAI-1 have been related prospectively to risk of recurrent myocardial infarction.⁷ The PAI-1 promoter has a common 4G/5G polymorphism 675 bp from the transcription start site that modulates in-vitro transcription and has an adjacent triglyceride responsive element.¹⁷ These observations lend support to the view that both genetic and environmental influences could regulate concentrations of PAI-1. Our heritability estimate is larger than the 42% estimated by Hong and colleagues¹⁰ in their 217 middle-aged and elderly twin pairs, and less than the 71% seen in a study of 25 twin pairs.¹² Other population studies have produced conflicting results; a French study reported that features of the insulin resistance syndrome are more important than genetic polymorphisms in determining PAI concentrations.²¹ The role of the PAI-1 gene in any observed heritability is also uncertain because at least two studies have reported that PAI-1 gene polymorphisms do not relate to concentrations,^{12,22} whereas some population studies indicate that the 4G/5G site alone accounts for up to 25% of variance.^{23,24} Some of these inconsistencies can be resolved by the suggestion of Cesari and colleagues, that additional, possibly still unidentified polymorphisms at the PAI-1 locus or other quantitative trait loci, must be responsible for genetic contribution to PAI-1 concentrations.¹²

The genetics of tissue plasminogen activator have not yet been properly assessed, although tissue plasminogen activator concentrations are known to relate to myocardial infarction.^{5,6} The heritability we reported for factor VII is similar to the 57% noted previously in 215 twin pairs.¹¹ In addition, known polymorphisms in the factor VII gene have been shown to contribute strongly to circulating concentrations, accounting for up to 40% of the total variance.²⁵

We reported high heritabilities for factor VIII and von Willebrand factor. Interestingly, analysis of the factor VIII promoter and 3' region²⁶ did not indicate any polymorphic sites that could account for this variation in

factor VIII concentrations. In the von Willebrand factor gene, 3' promoter polymorphisms have been described, which by haplotype account for up to 20% of the variability in concentration.²⁷ Both factor VIII and von Willebrand factor concentrations are regulated by a number of stimuli and to a large extent by blood group,¹³ with lowest concentrations in individuals with blood group O.²⁸ A small twin study¹³ recorded heritabilities of 57% for factor VIII and 66% for von Willebrand factor, and 30% of the genetic variance for von Willebrand factor was attributed to the effect of ABO blood type. The results of the present study suggest that most of the genetic effect on factor VIII and von Willebrand factor remains unexplained.

A common polymorphism in the factor XIII A-subunit (Val34Leu) has been investigated as a risk determinant of thrombosis in both arterial and venous systems.¹⁷ This polymorphism has a strong effect on factor XIII activation rates.²⁹ This functional effect can be detected with a factor XIII activity assay, whereas the polymorphism has little effect on protein concentrations as measured by ELISA.¹⁹ The differential effect of this genetic polymorphism on factor XIII function could be reflected by the much greater heritability of factor XIII activity than of the zymogen factor XIII A-subunit (which contains the active site of this enzyme) as measured by ELISA. Factor XIII B-subunit concentrations were much less defined by genetic factors than A-subunit concentrations. The B-subunit mainly serves as a carrier of the catalytic A-subunit in plasma.³⁰ Although all the A-subunit in the circulation is bound to B-subunit, there is a pool of around 40% B-subunit found uncomplexed in the circulation. Therefore variation in B-subunit concentrations buffered by the free pool could be less susceptible to evolutionary pressure and hence decreased heritability.

The finding of a common environmental influence for factor XIIa and XIII B-subunit in adult twins is difficult to explain. Adult twins do not share the environment to the extent they did when they shared the same home. However, some shared environmental factors might have a lasting effect. One example is the experience of an adverse environment in early life; there is evidence that environmental factors that affect fetal growth are related to increased concentrations of some haemostatic factors in adult life.³¹

The reciprocal activation between factor XII and kallikrein is at the crossroads of the coagulation, complement, kinin, and fibrinolysis systems. There is evidence to suggest that the activated form of factor XII as measured by ELISA is associated with the risk for coronary-artery disease.¹⁶ We therefore chose to analyse heritability of activated factor XII rather than zymogen. Our heritability estimate for activated factor XII is perhaps higher than anticipated. Low basal concentrations of activated coagulation factors, including activated factor XII, are generated continuously in physiological conditions, which increase in pre-thrombotic states.¹⁶ Whereas a high degree of heritability of circulating zymogen concentrations is readily understandable, it is more difficult to picture heritability of an activated factor. Perhaps the assay of activated factor XII correlated closely with concentrations of the factor XII zymogen, and heritability reflects that of the zymogen. Alternatively, there could be genetic regulatory elements in activation mechanisms of factor XII. A high degree of heritability of activated factor XII is however supported by evidence of an association between these concentrations and a common polymorphism in exon I of the factor XII gene.³²

Studies which have used family data to estimate genetic influences^{8,9} will have been confounded to some extent by cohort, age, and shared environmental effects. Only the twin design can discriminate between genetic and shared environment as sources of family resemblance and has the advantage of matching for age and (unknown) cohort effects.³³ Another advantage of studying twins rather than families is the possibility of extending the twin study with candidate-gene analysis. Since heritability is the total amount of population variance that can be explained by genetic effects it can be compared directly to the amount of variation due to specific polymorphisms. How much remains to be explained by as yet undetected loci is illustrated by a study of fetal haemoglobin concentrations.^{33,34} For example, the high heritability for factor XIII activity can probably be mostly explained by the strong effect of the factor XIII Val34Leu polymorphism on activity.¹⁹ Although the twin registry is limited to women for historical reasons, there is no reason to expect major differences between men and women and these results will be relevant to both sexes.

The moderate to large degree of heritability in ten key factors of fibrinolysis and coagulation we report indicates the importance of research into the genetic regulation of proteins involved in haemostasis and atherothrombotic disorders, including myocardial infarction and stroke.

Contributions

Marlies de Lange and Harold Snieder did the statistical analysis. Robert A S Ariens helped design the study, did the biochemical assays, and assessed quality control. Tim D Spector and Peter J Grant contributed to design of the study. All investigators contributed to writing and review of the manuscript.

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