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ORIGINAL ARTICLE

Heritability of plasma concentrations of clotting factors and measures of a prethrombotic state in a protein C-deficient family

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Summary. *Background*: Earlier studies found strong support for a genetic basis for regulation of coagulation factor levels and measures of a prethrombotic state (D-dimer, prothrombin fragment 1.2). *Objectives*: Estimation of how much of the variation in the levels of coagulation factors and measures of a prethrombotic state, including measures of protein C activation and inactivation, could be attributed to heritability and household effect. *Patients and methods*: Blood samples were collected from 330 members of a large kindred of French-Canadian origin with type I protein C deficiency. Heritability and common household effect were estimated for plasma concentrations of prothrombin, factor (F)V, factor VIII, factor (F)IX, fibrinogen, von Willebrand factor (VWF), antithrombin, protein C, protein S, protein Z, protein Z-dependent protease inhibitor (ZPI), fibrinopeptide A (FPA), protein C activation peptide (PCP), activated protein C–protein C inhibitor complex (APC–PCI), activated protein C–α_1-antitrypsin complex (APC–α1AT), prothrombin fragment 1.2 (F1.2) and D-dimer, using the variance component method in sequential oligo-genic linkage analysis routines (SOLAR). *Results*: The highest heritability was found for measures of thrombin activity (PCP and FPA). High estimates were also found for prothrombin, FV, FIX, protein C, protein Z, ZPI, APC–PCI and APC–α1AT. An important influence of shared household effect on phenotypic variation was found for VWF, antithrombin, protein S and F1.2. *Conclusions*: We found strong evidence for the heritability of single coagulation factors and measures of a prethrombotic state. Hemostatic markers with statistically significant heritability constitute potential targets for the identification of novel genes involved in the control of quantitative trait loci.

Keywords: coagulation, hereditary protein C deficiency, heritability.

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Introduction

Over a century ago, Virchow postulated that thrombosis was caused by alteration in the vessel wall, blood flow or the composition of the blood [1]. Several hereditary prothrombotic defects have been identified in the last four decades associated with a change in the composition of the blood. The first hereditary defects described were mutations in clot-preventing factors (antithrombin, protein C and protein S) [2–4]. In 1994 and 1996, two highly prevalent mutations, the factor (F)V Leiden mutation (FV G1691A) and the factor II G20210A mutation, were reported [5,6]. The latter two mutations are associated with resistance to inactivation of activated FV and elevated concentrations of prothrombin, respectively. Elevated concentrations of other procoagulant factors, such as fibrinogen, factor (F)VIII, factor (F)IX and factor (F)XI have been shown to increase the risk of venous thrombosis [7–10]. Several polymorphisms are known to influence plasma levels of fibrinogen [11]. For high levels of FVIII, evidence for a genetic determination by factors other than blood group and von Willebrand factor (VWF) has been found [12,13]. However, no polymorphisms have been discovered yet in the FVIII gene that can account for high levels of FVIII [14]. For FIX and FXI it is still unclear whether genetic factors contribute or not, and to what extent.

Recent studies have demonstrated a genetic basis for the regulation of plasma concentrations of coagulation factors and markers of a prethrombotic state [D-dimer and prothrombin activation fragment 1.2 (F1.2)] by estimating heritability within healthy individuals or relatives of individuals with arterial or venous thrombosis [15–18]. Previously, we published preliminary data demonstrating strong evidence for the heritability of levels of markers of protein C activation and inactivation in a large family from French Canadian descent with Type I protein C deficiency [19].

The present paper describes the finalized analysis on the heritability of levels of coagulation factors and measures of a prethrombotic state with the addition of important information on household influence. Heritability and common household effect were estimated for plasma concentrations of prothrombin, FV, FVIII, FIX, fibrinogen, VWF, antithrombin, protein C, protein S, protein Z, protein Z-dependent protease inhibitor (ZPI), fibrinopeptide A (FPA), protein C activation peptide (PCP), activated protein C–protein C inhibitor complex (APC–PCI), activated protein C–α_1-antitrypsin complex (APC–α1AT), F1.2 and D-dimer.

Materials and methods

Participants and inclusion criteria

Blood samples were collected from 330 members of a large kindred of French-Canadian origin with type I protein C deficiency, including spouses of family members with children. The ascertainment and evaluation of the family members was described previously [20]. All subjects completed questionnaires and were personally interviewed regarding their medical history in general, their risk factors for thrombosis (e.g. use of birth control pills, pregnancy, surgery, trauma, infection) and their thrombosis history. We classified a history of venous thrombosis as verified when subjects were hospitalized and treated for venous thrombosis with an objectively diagnosed deep venous thrombosis or pulmonary embolism. All participating subjects gave informed consent, or if individuals were under 18 years, a parent or legal guardian gave informed consent.

We excluded from all analyses women pregnant at the time of the blood draw ($n = 5$). We also excluded individuals using coumadin derivatives ($n = 15$) for vitamin K-dependent factors (including all measures of a prethrombotic state), as well as individuals with fibrinogen levels $< 100\,\mathrm{mg\,dL^{-1}}$ ($n = 3$) and FV levels $< 33\,\mathrm{U\,dL^{-1}}$ ($n = 1$). This study was approved by the Human Experimentation Committees of the University of Vermont College of Medicine, Burlington (VT, USA) and the Beth Israel Hospital, Boston (MA, USA).

Blood collection and processing

Peripheral blood was collected into siliconized glass Vacutainer tubes containing two different anticoagulants: 3.8% buffered citrate solution (Becton Dickinson, Franklin, NJ, USA), and SCAT-1 (25 μM PPACK, $200\,\mathrm{kIU\,mL^{-1}}$ aprotinin, 4.5 mM EDTA; Hematologic Technologies, Essex, VT, USA). Platelet-poor plasma from freshly drawn whole blood was produced within 1 h by centrifugation at $3000 \times g$ for 10 min at room temperature and stored at −70 °C. Frozen plasma samples were thawed at 37 °C just before assay performance.

Assay methodology

All assays were performed in the investigators' laboratories either by ELISA: FV antigen [coefficient of variation (CV) 5.8%] [21], FVIII antigen (CV 7.8%), FIX antigen (CV 10%), VWF (CV 3%) [22], protein Z (CV 6.5%) [24], ZPI (CV 7.2%) [24], APC–α1AT complex (CV 12.4%) [25] and APC–PCI complex (CV 11.7%) [25], or radioimmunoassay: prothrombin (CV 8%) [26], antithrombin (CV 5%) [27,28], protein S (CV 9.8%) [29], F1.2 (CV 8%) [28], PCP (CV 14%) [28] and FPA (CV 8%) [28], the Clauss method for fibrinogen (CV 1.7%) [30,31] using the ST4 instrument (Diagnostica Stago, Parsipanny, NJ, USA), a clot-based functional assay for protein C: (CV 5.5%) [20,23] or micro latex bead agglutination for D-dimer (CV 9.2%) (Biomerieux, Durham, NC, USA) [32,33].

FV antigen analysis was performed with an in-house assay [21]. The kits for FVIII antigen, FIX antigen, protein C and VWF were provided by Diagnostica Stago, and assays were performed following the manufacturers' instructions. The assays for APC–PCI complex and APC–α1AT complex were performed using a commercially available assay at Affinity Biologicals Inc. (Ancaster, Ontario, Canada).

The number of individuals per assay varied, based on the availability of appropriate stored samples from 83 to 287.

Statistical analysis

To reduce skewness and kurtosis, we applied log transformation to the levels of FV, VWF, antithrombin, FPA, PCP, APC–α1AT complex, APC–PCI complex and D-dimer, square root transformation to the levels of FVIII antigen and F1.2, and reciprocal transformation to the levels of FIX antigen.

Heritability, the proportion of the phenotypic variance attributed to polygenes, and common household effect, the proportion of the variance attributed to environmental factors shared within a household, were estimated for each variable using the variance component method in SOLAR [34]. In addition, we studied the effect on the heritability and household estimates of excluding individuals with a definite venous thrombotic history, the protein C3363C insertion and the prothrombin G20210A mutation. As only three family members carried the FV Leiden mutation, we did not study the effect of excluding individuals with this mutation. The distribution of each (transformed) variable was assumed to be multivariate normal with a variance–covariance matrix following the formula: covariance (one person to another person) $= h^2K + c^2H + e^2I$, with K derived from the kinship matrix, H from the household matrix and I from the identity matrix. The additive genetic and household components of variance were estimated using maximum

likelihood analysis. The adjustment for covariates was made as part of the heritability analysis. All analyses were adjusted for age and sex. We also adjusted for the use of oral contraceptives or hormone replacement therapy for prothrombin, FV, FVIII antigen, fibrinogen, VWF, protein S and C, antithrombin, and all measures of a prethrombotic state. In addition, levels of VWF were adjusted for ABO blood group and levels of FVIII antigen were adjusted for ABO blood group and in some analyses for VWF.

Results

The mean age of the 322 family members at the blood draw was 31 years (range 1–90), 131 (41%) were men and the mean body mass index ($kg\,m^{-2}$) was 24.7 (range 12–52). The main characteristics of the 322 family members are outlined in Table 1. In total, 25% carried the 3363C insertion in the protein C gene, 13% carried the prothrombin G20210A polymorphism and 1% carried the FV Leden mutation, as confirmed by individual genotyping, and 9% had experienced a definite venous thrombotic event. The mean level of prothrombin was $128.4\,U\,dL^{-1}$ in individuals with the G20210A mutation (range $90.7–165.0\,U\,dL^{-1}$).

Table 2 shows the mean level, standard deviation and range of all coagulation factors and measures of a prethrombotic state. The mean level and 95% confidence interval ranges of levels of coagulation factors and measures of a prethrombotic state were largely in accordance with normal ranges found in our laboratory or by others [20,35,36]. We found slightly high levels for D-dimer in seven family members. For FVIII antigen levels we found a broad range, but most individuals with lower levels of FVIII had correspondingly low levels of VWF and tended to have blood type O.

Table 1 Main characteristics of all subjects

Characteristics at blood draw* ($N = 322$)	
Age (years; range)	31.3 (1–90)
Number of households	181
Mean number of individuals per household (range)	1.8 (1–6)
Spouses	21
Sex (M/F)	131/191
Body mass index ($kg\,m^{-2}$; range)	24.7 (12–52)
Smokers (age >13 years)	72/246 (29%)
Personal history of venous thrombosis (possible and definite events)	48/322 (15%)
Personal history of venous thrombosis (definite events)	28/322 (9%)
Anticoagulation treatment	15/322 (5%)
Hormone replacement therapy	10/181 (6%)
Oral contraceptive use	18/183 (10%)
Blood group O	80/290 (28%)
Protein C 3363C insertion	79/317 (25%)
Prothrombin G20210A mutation	38/293 (13%)†
Factor V Leiden mutation	3/302 (1%)

*Except for age, sex and body mass index, all characteristics are given as the number and percentage of individuals with the characteristic, including the number of individuals for whom information was available. †One individual was homozygous for the prothrombin G20210A mutation.

Heritability and household estimates of single coagulation factors or markers

Table 3 shows heritability and household effect estimates for all coagulation factors and measures of a prethrombotic state. Among procoagulant factors, we found high heritability estimates for prothrombin (69.6%), FV (71.4%) and FIX antigen (50.3%) and lower heritability for fibrinogen (29.7%), VWF (25.3%), and FVIII antigen when adjusted for VWF (19.5%).

Table 2 Mean levels, standard deviation and range of all analytes

Analytes (units)	*N*	Mean	SD	Range
Prothrombin ($U\,dL^{-1}$)	164	104.91	20.38	71.00–165.00
Factor V ($U\,dL^{-1}$)	259	117.75	37.13	54.76–299.75
Factor VIII antigen ($U\,dL^{-1}$)*	198	116.98	42.06	44.88–250.00
Factor IX antigen ($U\,dL^{-1}$)	222	93.08	30.69	52.87–250.00
Fibrinogen ($mg\,dL^{-1}$)	283	273.08	67.78	140.00–573.00
VWF ($U\,dL^{-1}$)	236	115.99	45.92	31.45–300.00
Antithrombin (% of normal)	176	98.23	16.52	64.10–161.60
Protein C (% of normal)	267	90.16	31.30	16.00–203.00
Protein C (3363C insertion only; % of normal)	59	50.68	15.11	16.00–109.00
Protein S (total; $\mu g\,mL^{-1}$)	163	15.35	2.48	8.00–24.10
Protein Z (% of normal)	274	104.50	36.02	21.00–237.00
ZPI (% of normal)	287	100.33	29.96	30.00–215.00
FPA (nM)	147	1.43	1.03	0.26–6.65
PCP ($pmol\,L^{-1}$)	83	1.33	0.74	0.25–4.17
APC–α1AT complex (nM)	164	0.28	0.49	0.01–4.30
APC–PCI complex (nM)	164	0.06	0.06	0.01–0.43
F1.2 ($nmol\,L^{-1}$)	147	2.28	1.56	0.40–10.20
D-dimer ($ng\,mL^{-1}$)	263	154.40	281.18	4.82–2551.48

VWF, Von Willebrand factor; ZPI, protein Z-dependent protease inhibitor; FPA, fibrinopeptide A; PCP, protein C activation peptide; APC–α1AT, activated protein C–α_1-antitrypsin; APC–PCI, activated protein C–protein C inhibitor; F1.2, prothrombin fragment 1.2. *Only individuals for whom blood type was known.

Table 3 Proportion of phenotypic variance explained by covariates, heritability (h^2) and household effect (c^2)

Analytes	*N*	Proportion of variance, %				
		Covariates	h^2	SE (h^2)	c^2	SE (c^2)
Prothrombin	164	11.7	69.6*	18.1	4.1	15.0
Factor V	259	14.8	71.4*	13.3	2.8	9.5
Factor VIII antigen (adjusted for VWF)	187	46.1	19.5*	15.9	6.6	10.9
Factor VIII antigen	198	26.9	30.9*	15.2	0.0	N/A†
Factor IX antigen	222	11.3	50.3*	19.0	3.3	12.0
Fibrinogen	283	24.3	29.7*	13.6	0.0	N/A†
VWF	236	19.9	25.3*	16.4	30.7*	11.1
Antithrombin	176	23.1	6.1	13.4	33.5*	13.9
Protein C	267	3.3	40.6*	15.8	4.4	10.3
Protein S (total)	163	23.7	10.5	31.1	36.9*	21.7
Protein Z	274	0.0	66.7*	12.6	6.6	8.8
ZPI	287	7.4	42.8*	15.8	1.3	9.6
FPA	147	0.0	92.0*	15.9	0.0	N/A†
PCP	83	5.6	75.4*	29.6	0.0	N/A†
APC–α1AT complex	164	1.8	58.6*	22.3	4.8	14.2
APC–PCI complex	164	0.0	58.4*	18.0	0.0	N/A†
F1.2	147	29.6	22.1	20.3	44.1*	13.3
D-dimer	263	13.5	6.6	12.9	5.2	10.3

SE, standard error; VWF, von Willebrand factor; ZPI, protein Z-dependent protease inhibitor; FPA, fibrinopeptide A; PCP, protein C activation peptide; APC–α1AT, activated protein C–α_1-antitrypsin; APC–PCI, activated protein C–protein C inhibitor; F1.2, prothrombin fragment 1.2. *Significant at $P < 0.05$. †No standard errors can be approximated for estimates of 0% or 100% in likelihood analysis.

Interestingly, a household effect was found for VWF (30.7%), but not for FVIII antigen (unadjusted for VWF) despite the relationship between FVIII and its carrier protein VWF. Significant heritability estimates for anticoagulant factors were found for protein C (40.6% for all tested individuals; 38.5% excluding those with the 3363C insertion, and 5.2% in those with the 3363C insertion), protein Z (66.7%) and ZPI (42.8%). The estimates of heritability were low for antithrombin (6.1%), and protein S levels (10.5%), while for both substantial household effects were found (respectively 33.5% and 36.9%). For measures of the activation and inactivation of protein C, high heritability estimates were found for PCP (75.4%) and the APC-inhibitor complexes (58.6% for APC–α1AT and 58.4% for APC–PCI). FPA showed a high heritability (92.0%), probably reflecting the activity of thrombin. Heritability estimates, however, were low for prothrombin activation (F1.2; 22.1%) and D-dimer levels (6.6%), although for F1.2 a high household effect was found (44.1%).

No major differences (>30%) in heritability estimates or household estimates were seen after excluding family members with a definite history of venous thrombosis. However, we did find an increase or decrease of >30% in the heritability or household effects of several analytes after excluding individuals with the 3363C protein C gene insertion or the prothrombin G20210A mutation. Excluding individuals with the 3363C protein C mutation increased heritability and decreased the household effect for antithrombin to, respectively, 41% and 2.5%, and increased the household effect of prothrombin to 36%. Excluding individuals with the prothrombin G20210A mutation increased heritability for prothrombin to 28% and for APC–AT to 89%.

Discussion

This study was performed to establish evidence for a genetic basis for plasma concentrations of hemostatic markers known to confer risk of thrombosis. The highest heritability estimates were found for the measures of thrombin activity (PCP and FPA). These constitute the best potential targets for the identification of novel genes involved in the control of quantitative trait loci. High heritability was also found for prothrombin, FV, FIX antigen, protein C, protein Z, ZPI, APC–PCI and APC–α1AT. Fibrinogen, VWF, antithrombin, thrombin generation (F1.2) and endogenous fibrinolysis (D-dimer) showed low heritability. Heritability was also low for protein S; however, protein S differs from the other vitamin K-dependent factors in that it is not a protease and is 60% bound to C4b-bp in plasma. The heritability estimate for FVIII antigen was low after adjustment for VWF levels, probably due to the dependance of FVIII levels on the stabilization of FVIII in plasma mediated by FVIII binding to VWF. For protein C we found a heritability of 40.6% for all tested individuals, a heritability of 38.5% after excluding those with the 3363C insertion, and a heritability of 5.2% in those with the protein C 3363C insertion. The latter suggests that due to the lower sample size and having one active allele instead of two, by chance, other genes affecting protein C may not be present or may not affect individuals with lower levels of protein C.

An important influence of the shared household effect on phenotypic variation was found for VWF, antithrombin, protein S and F1.2. A household-specific acute or chronic activation of the coagulation pathway by tobacco use, diet, physical activity or other shared environmental characteristics could explain the

high household effects not only for F1.2 and VWF, but also for the anticoagulation factors protein S and antithrombin, as household-specific differences in activation of the clotting system may give rise to compensatory differences in these inhibitors.

Several other studies have estimated heritability with regard to the coagulation system, but focused primarily on procoagulant and anticoagulant factors [15–18]. We found high heritability for markers of thrombin activity (FPA and PCP) and the activated protein C-inhibitor complexes. In contrast, F1.2, a marker of thrombin generation, did not demonstrate significant heritability but did show a significant household effect. Adjustment for F1.2 in the present study did not change the heritability of FPA, PCP and the activated protein C-inhibitor complexes. Thus, thrombin generation, a critical step in hemostasis, seems to be influenced largely by environmental factors. However, its activity as measured by FPA, PCP and complexes of activated protein C with its inhibitors appears to be tightly controlled through genetic mechanisms which may implicate proteins like thrombomodulin, the endothelial cell protein C receptor and fibrinogen. Table 4 gives a comparison of the heritability estimates of the most recently published studies and the present study [15–18]. The variance component method used to estimate heritability and household effect assesses the relative role of genetic and environmental causes of variation of a quantitative trait in a particular setting and population. Because genes and environment are different in various populations, heritability estimates cannot be readily compared quantitatively across populations. The populations shown in Table 4 are different not only by geography but also by selection of the sample from the population: De Souto *et al*. [15] estimated heritability in family members of probands from multiple families with hereditary thrombophilia in Catalonia, Spain, whereas the studies by Ariëns *et al*. and De Lange *et al*. [16,17] comprised healthy twins from the UK. We studied a single kindred, which decreases both the genetic variance, due to familial genetic similarity, and the random environmental variance, due to greater similarity of lifestyles among different households within a family. Although the total variance is generally smaller in a single kindred compared with more varied populations, the increased genetic homogeneity facilitates the likelihood of detecting the gene(s) underlying the observed heritability. A potential source of error across populations could be attributed to the methods used to measure analytes with regard to the variation in preanalytical and analytical errors, which could decrease heritability [37]. We did not account for non-additive sources of genetic variance such as dominance and epistasis, which could also decrease heritability.

Table 4 Heritability estimates (h^2, %) found in the present study and other studies

	h^2, %			
Analyte	USA	UK-1[16,17]	UK-2[18]	Spain[15]
Prothrombin	70	57	ND	49
Factor V	71	ND	ND	44
Factor VIII antigen	20*	61†	ND	40†
Factor IX antigen	50	ND	ND	39
Fibrinogen	30	44	35	34
VWF	25	75	ND	32
Antithrombin	6	ND	ND	49
Protein C	41	ND	ND	50
Protein S	11	ND	ND	46
Protein Z	67	ND	ND	ND
ZPI	43	ND	ND	ND
FPA	96	ND	ND	ND
PCP	75	ND	ND	ND
APC–α1AT complex	59	ND	ND	ND
APC–PCI complex	58	ND	ND	ND
F1.2	22	45	ND	ND
D-dimer	7	65	ND	11

VWF, Von Willebrand factor; ZPI, protein Z-dependent protease inhibitor; FPA, fibrinopeptide A; PCP, protein C activation peptide; APC–α1AT, activated protein C–α1-antitrypsin; APC–PCI, activated protein C–protein C inhibitor; F1.2, prothrombin fragment 1.2; ND, not determined.
*Adjusted for ABO blood group and VWF. †Not adjusted for ABO blood group and VWF.
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The heritability estimates in this paper are only slightly different from the estimates we published earlier [19], and differ mainly due to the estimation of household effects. A shared household effect apparently seems to explain a part of the heritability previously published for levels of VWF, antithrombin and protein S [19], showing the importance of estimating household effects as part of a variance component analysis. Differences between studies in Table 4 could thus also be attributable to the magnitude of the household effect.

Exclusion of individuals with the 3363C insertion or the prothrombin G20210A mutation influenced few heritability and household estimates of coagulation factors or activation markers. An increase in the household effect after removal of carriers of a certain gene or trait could be the result of a decrease in the total variance, so that the household component accounts for more of the total variance. We have no explanation for the increase in heritability estimates after removal of carriers of a gene or trait other than that the inheritance of other genes fitted the polygenic model better once the gene-carriers were removed. However, these changes can be used in defining the most informative subsets in subsequent quantitative trait loci linkage analysis.

In conclusion, we found strong evidence for the heritability of single coagulation factors and measures of a prethrombotic state. Hemostatic markers with statistically significant heritability constitute potential targets for the identification of novel genes involved in the control of quantitative trait loci [38,39].

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