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Genetic Susceptibility to Thrombosis and Its Relationship to Physiological Risk Factors: The GAIT Study

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Although there are a number of well-characterized genetic defects that lead to increased risk of thrombosis, little information is available on the relative importance of genetic factors in thrombosis risk in the general population. We performed a family-based study of the genetics of thrombosis in the Spanish population to assess the heritability of thrombosis and to identify the joint actions of genes on thrombosis risk and related quantitative hemostasis phenotypes. We examined 398 individuals in 21 extended pedigrees. Twelve pedigrees were ascertained through a proband with idiopathic thrombosis, and the remaining pedigrees were randomly ascertained. The heritability of thrombosis liability and the genetic correlations between thrombosis and each of the quantitative risk factors were estimated by means of a novel variance component method that used a multivariate threshold model. More than 60% of the variation in susceptibility to common thrombosis is attributable to genetic factors. Several quantitative risk factors exhibited significant genetic correlations with thrombosis, indicating that some of the genes that influence quantitative variation in these physiological correlates also influence the risk of thrombosis. Traits that exhibited significant genetic correlations with thrombosis included levels of several coagulation factors (factors VII, VIII, IX, XI, XII, and von Willebrand), tissue plasminogen activator, homocysteine, and the activated protein C ratio. This is the first study that quantifies the genetic component of susceptibility to common thrombosis. The high heritability of thrombosis risk and the significant genetic correlations between thrombosis and related risk factors suggest that the exploitation of correlated quantitative phenotypes will aid the search for susceptibility genes.

Introduction

Thrombosis is a common cause of morbidity and mortality in industrialized nations. Both venous and arterial forms of thrombosis are of great public-health importance. Although there is little direct information on prevalence, retrospective and prospective data (Coon et al. 1973; Anderson et al. 1991; Nordstrom et al. 1992) suggest a minimum lifetime prevalence of 5%–10% for deep-vein thrombosis. After the inclusion of arterial thromboses, other venous thromboses, and undiagnosed thrombotic conditions, the true lifetime prevalence of thrombosis must be substantially >10%.

The canonical causes of thrombosis include both environmental and genetic factors (Rosendaal 1999). The high prevalence of thrombosis and its known environmental influences, such as smoking and oral contraceptive use, suggest that multiple genes of varying effects will be involved in determining susceptibility to thrombosis. Such complex oligogenic inheritance is also likely to involve gene-gene and gene-environment interactions (Hasstedt et al. 1998). Although there are a number of well-characterized genetic defects that lead to increased thrombotic risk (Lane et al. 1996), it is unlikely that these comparatively infrequent mutations constitute the primary genetic influences on risk of common late-onset thrombosis. In fact, very little information is available on the relative importance of genetic factors in thrombosis risk in the general population. Because of the paucity of family-based studies, there are no extant estimates of the heritability of thrombosis risk.

The physiological cascade that underlies the normal formation of thrombin and the pathological endpoint of thrombosis is complex, with many components involved in the coagulation and fibrinolytic pathways. The identification of quantitative risk factors for thrombosis has accelerated in recent years. Numerous hemostatic factors—including fibrinogen, factor VII, factor VIII, von Willebrand factor, and homocysteine—have been implicated as possible concomitants of both

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venous (Koster et al. 1994, 1995; MacCallum et al. 1995; den Heijer et al. 1996) and arterial thrombosis (Meade et al. 1986; Hamsten et al. 1987; Ernst and Resch 1993; Ridker et al. 1993; Folsom et al. 1997; Nygard et al. 1997). Regardless of their causal relationships with thrombosis, such correlated phenotypes can provide additional information about the genetic basis of thrombosis risk. Recent advances in statistical genetics allow the simultaneous examination of the genetic and environmental sources of correlations between such continuous physiological measures and discrete disease outcomes (Williams et al. 1999*b*) through the examination of data from large families. Such approaches, when coupled with modern molecular genetic technologies, will soon permit the localization and identification of the quantitative trait loci (QTLs) that underlie thrombosis risk. Prior to embarking on the potentially expensive search for the actual loci involved, it is prudent to evaluate the magnitude of genetic effects on thrombosis and to test for the pleiotropic effects of genes on both risk factors and disease.

As a first step toward the ultimate goal of the identification of novel genes involved in thrombosis susceptibility, we performed a family-based study of the genetics of thrombosis in the Spanish population. This study design has allowed us to quantify the heritability of thrombosis and to identify the joint actions of genes on thrombosis risk and a number of related quantitative phenotypes. Previous analyses of these 27 quantitative phenotypes have already demonstrated strong heritabilities for most of these traits (Souto et al. 2000). The majority of the heritabilities ranged between 0.22 and 0.55, with somewhat higher values seen for factor XII (0.67), activated protein C resistance ratio (0.71), and activated partial thromboplastin time (0.83), and somewhat lower values observed for D-dimer (0.11) and tissue factor (0.17).

Subjects and Methods

Study Population and Diagnosis

The Genetic Analysis of Idiopathic Thrombophilia (GAIT) Study is composed of 21 extended families, 12 of which were ascertained through a proband with thrombophilia and 9 of which were obtained randomly. Thrombophilia was defined as multiple thrombotic events (at least one of which was spontaneous), a single spontaneous episode of thrombosis with a first-degree relative also affected, or onset of thrombosis at age <45 years. Ten of the 12 thrombophilic probands had onset at age <45 years, 8 had multiple episodes of thrombosis, and 2 probands were ascertained on the basis of family history. Diagnoses of the thrombophilic probands were verified by objective methods. Thrombosis in these individuals was considered idiopathic because of exclusion of all biological causes of thrombosis, including antithrombin deficiency, Protein S and C deficiencies, activated protein C resistance, plasminogen deficiency, heparin cofactor II deficiency, Factor V Leiden, dysfibrogenemia, lupus anticoagulant, and antiphospholipid antibodies, known at the time of recruitment (1995–97).

A total of 398 individuals (with a mean of 19 individuals per family) were examined. Most pedigrees contained three generations, although eight families had four generations and one family had five. Subjects had a mean age at examination of 37.7 years, and there were approximately equal numbers of males and females. The composition of the families and the collection of lifestyle, medical, and family-history data have been described elsewhere (Souto et al. 2000). Reported history of thrombosis in family members was verified by examination of medical records, when available. Although some deceased family members had a history of thrombosis, only individuals interviewed and examined in person were included in the analyses. The primary residence of each subject was also determined, to assess the contribution of shared environmental influences (such as diet) common to members of a household. The study was performed according to the Declaration of Helsinki of 1975, and all adult patients provided informed consent for themselves and for their minor children.

Laboratory Measurements and Techniques

A total of 27 quantitative phenotypes were measured in the plasma of each individual. None of the participants was being treated with anticoagulant therapy at the time of blood drawing. Activated partial thromboplastin time (APTT), prothrombin time (PT), coagulation factors (FII, FV, FVII, FVIII, FIX, FX, FXI, and FXII), functional protein S, and the activated protein C-sensitivity ratio (APCR) were measured by automated coagulometry. Antithrombin, protein C, heparin cofactor II, plasminogen, and plasminogen activator inhibitor were measured by chromogenic methods. Fibrinogen was measured by the Clauss method (Clauss 1957). Total and free protein S, tissue plasminogen activator (t-PA), D-dimer (DD), tissue factor (TF), and von Willebrand factor (vWF) were assayed by use of commercially available ELISA kits. Histidine-rich glycoprotein (HRG) was measured by electroimmunoassay, tissue factor pathway inhibitor (TFPI) by a functional method (Sandset et al. 1991), and homocysteine by a fluorimetric method (Hyland and Bottiglieri 1992). ABO blood groups and Factor V Leiden genotypes were assessed by means of standard techniques. Details of phenotype assays are available in Souto et al. (2000).

Statistical Genetic Analysis

The heritability (the proportion of the total phenotypic variability attributable to genetic effects) of susceptibility to thrombosis was evaluated by means of a pedigree-based maximum-likelihood method that models affection status as a threshold process (Duggirala et al. 1997, 1999*a*; Williams et al. 1999*b*). Although disease status is usually operationalized as a discrete trait, with individuals scored as unaffected or affected, it is generally assumed that there is an unobservable continuous trait, termed "liability" or "susceptibility," that determines affection status. If an individual's liability score exceeds some specified threshold, disease results; if it is below the threshold, the individual is unaffected. The threshold is placed in an age- and sex-specific manner, to produce the appropriate population prevalence. A specific individual's liability is only known to be above or below the threshold, depending on the individual's affection status, and an integral over the appropriate region of the curve is used to estimate each person's liability value. Since such continuous processes determine most biological phenomena, it is useful to make inferences on the underlying continuous scale, which is more consistent with current models of gene action. Threshold models permit such inferences regarding the latent underlying quantitative scale to be made. To use a threshold model, some weak assumptions regarding the form of the underlying continuous process are necessary. For genetic modeling, we assume that the underlying liability distribution is normal, and we calculate the joint probability of observing the disease statuses of family members by using a multivariate normal distribution that allows for correlations among family members.

The analysis of heritability of thrombosis susceptibility was performed using the variance component method. The total phenotypic variance in thrombosis susceptibility was partitioned into three components: (1) an additive genetic variance, caused by the sum of the average effects of all the genes that influence thrombosis; (2) a shared environmental variance, caused by the effects of environmental factors that are common to households; and (3) a random environmental variance specific to each individual. The random environmental variance also absorbs nonadditive genetic effects, such as interactions between alleles within loci (dominance effects), interactions between alleles at different loci (epistatic effects), and effects caused by gene-environment interactions. Therefore, such models will generally underestimate the role of genetics in the determination of the trait.

With this approach, the relative components of variance can be estimated by use of maximum-likelihood estimation. Evaluation of the likelihood function for a pedigree involves high dimensional integration of a multivariate normal distribution. The limits of integration may be different for each individual, depending on affection status as well as on any covariates that are introduced as fixed effects in the model for the mean liability. In the current analyses, these covariates included age and sex.

To study the genetic relationships between thrombosis susceptibility and quantitative variation in hemostatic parameters, we used a new mixed discrete/continuous trait variance component analysis (Williams et al. 1999*b*). This analysis used a modified variance component method to accommodate a mixture of discrete and continuous data and allows the phenotypic correlations between these traits to be decomposed into factors caused by common genetic influences and common environmental influences on the two traits. Examination of the underlying determinants of phenotypic correlations provides information on the role of pleiotropic genetic effects.

All the extant epidemiological evidence for the relationship between thrombosis and hemostatic parameters is based on the evaluation of phenotypic correlations. However, the decomposition of phenotypic correlations into genetic and environmental components is potentially valuable, since hidden relationships between traits can be revealed (Comuzzie et al. 1996). For example, if trait $y_1 = g_1 + e_1$ and trait $y_2 = g_2 + e_2$, where g and e denote genetic and environmental effects, the observed correlations between the phenotypic traits are determined by the latent genetic and environmental correlations between the component variables. By studying both traits in extended families, we can estimate both the genetic (ρ_g) and the environmental (ρ_e) correlations between traits. The phenotypic correlation (ρ_p) is derived from these two constituent correlations and the heritabilities of the traits:

$$\rho_p = \sqrt{(h_1^2 h_2^2)}\rho_g + \sqrt{(1 - h_1^2)}\sqrt{(1 - h_2^2)}\rho_e .$$

We have incorporated the threshold model (Duggirala et al. 1997, 1999*a*) and the mixed discrete/continuous trait variance component method (Williams et al. 1999*b*) into our statistical genetic computer package, *SOLAR* (Almasy and Blangero 1998). All statistical genetic analyses were performed using *SOLAR,* with these modifications. Estimates of variance component parameters, including the heritabilities of thrombosis and the quantitative measures and all the phenotypic, genetic, and environmental correlations between thrombosis and the quantitative phenotypes, were obtained by use of maximum-likelihood estimation. All hypothesis tests were performed using likelihood-ratio test statistics (Kendall and Stuart 1972; Self and Liang 1987).

Because 12 of the 21 pedigrees were ascertained

through a thrombophilic proband, all analyses included an ascertainment correction, to allow unbiased estimation of parameters relevant to the general population. To achieve this, the likelihood for each family ascertained through a thrombophilic proband was conditioned on the phenotype of the proband (Hopper and Mathews 1982; Boehnke and Lange 1984). Since two families were ascertained, in part, because of the family history of the proband, analyses were repeated conditioning on both the original proband and the affected first-degree relative in these two families. However, the results of the analyses were unchanged.

Results

Characteristics of Affected Individuals

A total of 53 people with venous or arterial thrombosis were identified, 47 in the families ascertained through thrombophilic probands and 6 in the randomly ascertained families. The number of affected individuals per family ascertained through a thrombophilic proband was 2–8, with a mean of 3.9. The distribution of thrombotic subjects in these extended families included many instances of affected first-degree relatives (siblings or parents and children) but also grandparents, aunts or uncles, and first cousins. Eight of these families contained cases of both arterial and venous thrombosis. Two of the randomly ascertained families each had two individuals with thrombophlebitis. One of these was a parent-child pair, but the other consisted of two unrelated individuals (in-laws). One randomly ascertained family had a single individual with deep-vein thrombosis, and one had an individual with transient ischemic attacks.

There were slightly more affected females ($n = 31$, 58.5%) than males ($n = 22$, 41.5%), and the age at diagnosis of first thrombosis was 12–76 years, with a mean of 44.5 (table 1). When venous and arterial thrombosis were considered separately, 40 individuals, with an average age at first diagnosis of 39.7 years, had one or more diagnoses of venous thrombosis; 17 individuals, with an average age at first diagnosis of 61.0 years, had one or more arterial thromboses. The early observed age at diagnosis for venous thrombosis is partially a function of the ascertainment criteria. Deep-vein thrombosis was the most common condition ($n = 28$) and superficial thrombophlebitis (SFT) the second most common ($n = 14$). Fifteen (28%) of the 53 affected people had multiple thrombotic diagnoses, and five (9.4%) of these people had both venous and arterial events. Twelve individuals had deep-vein thrombosis and one to three other venous or arterial thromboses; one person had ischemic stroke and transient ischemic attacks; one person had SFT and pulmonary embolism; and one had SFT and other venous thrombosis.

Table 1

Number and Percent of Individuals in Each Diagnostic Category of Thrombosis and Age at Diagnosis

Diagnosis	No. (and %) of Individuals with Thrombosis	Mean Age at Diagnosis (years)
Venous thrombosis:		
Deep-vein thrombosis	28 (52.8)	40.3
Pulmonary embolism	9 (17.0)	45.6
SFT	14 (26.4)	41.2
Other venous thrombosis	3 (5.7)	58.0
Any venous thrombosis	40 (75.5)	39.7
Arterial thrombosis:		
Myocardial infarction	4 (7.5)	66.5
Angina pectoris	4 (7.5)	57.3
Ischemic stroke	6 (11.3)	61.0
Transient ischemic attack	5 (9.4)	55.4
Any arterial thrombosis	17 (32.1)	61.0
Any thrombosis	53 (100.0)	44.5

NOTE.—Some individuals are represented in multiple diagnostic categories.

Genetic Determinants of Liability to Thrombosis

The evidence for a strong genetic influence on risk of thrombosis was striking. Liability to thrombosis exhibited an additive genetic heritability of 0.61 ± 0.16 ($P = 9 \times 10^{-5}$), indicating that, after correction for the effects of age and sex, 61% of the variation in liability to thrombosis at the population level can be attributed to genetic factors. No shared environmental effects were found among members of a household for liability to thrombosis. Therefore, the above heritability estimates are unlikely to be inflated by nongenetic correlations among family members, and environmental factors shared by members of a household, such as diet, do not have major effects on thrombosis susceptibility. When the diagnoses considered are restricted to venous thrombosis, excluding arterial thrombotic events, the additive genetic heritability is not significantly different from that obtained with any thrombosis. Similarly, when venous and arterial thrombosis are analyzed jointly as two distinct traits, the phenotypic correlation between these two manifestations of thrombosis is .333 ($P = .0126$), and the genetic correlation is .55 ($P = .09$). Additionally, the genetic correlation is not significantly different from 1. Both the robustness of the heritability when combining across venous and arterial diagnoses and the fact that the genetic correlation is not significantly different from one strongly suggest that arterial and venous thromboses are highly genetically correlated and that our broad phenotypic characterization will be useful to increase the power to detect genetic effects.

Correlations between Thrombosis Liability and Quantitative Risk Factors

Table 2 shows the results of bivariate genetic analyses of thrombosis, with each of the quantitative physiological traits considered. Only the nine quantitative traits showing at least one significant correlation ($P < .05$) are presented. Of these, seven exhibit significant phenotypic correlations with thrombosis susceptibility, eight demonstrate significant genetic correlations with thrombosis, and only two exhibit significant environmental correlations. The largest phenotypic correlations ($|\rho_p| > 0.2$) are seen between FVIII, vWF, APCR, FXI, homocysteine, and thrombosis.

The genetic correlations provide strong evidence for significant pleiotropy underlying the covariation between several of the quantitative traits and thrombosis risk. Those quantitative measures exhibiting the largest genetic correlations ($|\rho_g| > 0.6$) with thrombosis include vWF, t-PA, FVIII, homocysteine, and APCR. The only traits to exhibit significant environmental correlations with thrombosis were APCR and FVII. Table 2 provides a good demonstration of how low-phenotypic correlations may misrepresent the true underlying relationships. Both FIX and FVII failed to show significant phenotypic correlations with thrombosis. However, both provide strong evidence for correlations between genetic effects (FIX) and environmental effects (FVII) with thrombosis. Similarly, the genetic and environmental correlations between APCR and thrombosis are of similar magnitudes but exhibit different directions. When such differences in sign appear, the phenotypic correlation is attenuated, although the underlying components suggest much stronger correlations. Relationships between APCR and thrombosis were unchanged when the presence of the Factor V Leiden mutation (there were nine heterozygotes in the sample) was statistically controlled. Similarly, the correlations between FVIII and thrombosis were unchanged when ABO blood type was incorporated into the model.

Table 2

Phenotypic, Genetic, and Environmental Correlations of Quantitative Risk Factors with Thrombosis

Phenotype[a]	ρ_p	P[b]	ρ_g	P	ρ_e	P[b]
APCR	−.230	.0003	−.650	1×10^{-6}	.669	.0006
FVII	.025	NS	−.354	.0564	.568	.0091
FVIII	.288	.0002	.689	.0005	−.126	NS
FIX	.151	.0787	.597	.0131	−.198	NS
FXI	.209	.0180	.564	.0245	.070	NS
FXII	.172	.0339	.351	.0500	−.145	NS
Homocysteine	.227	.0018	.652	.0015	−.028	NS
t-PA	.180	.0002	.752	.0070	−.099	NS
vWF	.261	.0010	.729	.0005	−.181	NS

[a] Only phenotypes with one or more correlations having $P < .05$ are shown.

[b] NS = nonsignificant ($P > .10$).

Discussion

This is the first study that formally documents the large genetic component for risk of thrombosis. By gathering and analyzing data on extended pedigrees that have been methodically ascertained to allow general population inferences, we have begun to fill a critical gap in the study designs used in thrombosis genetics. Researchers in hemostasis/thrombosis generally have not actively pursued family studies, except for the occasional serendipitous collection of unusual families with high densities of affected individuals. Therefore, most of our knowledge regarding the genetic factors involved in common thrombosis has been limited to association studies that use case-control designs to look at known polymorphic variations in candidate genes (Poort et al. 1996; Rosendaal 1997; Rosendaal et al. 1997; Iacoviello et al. 1998). Although such studies provide important indirect evidence for the presence of genetic effects, they have a number of weaknesses. These include their limitation to known candidate genes, their propensity for type I errors caused by hidden population stratification, the lack of direct evaluation of familial transmission, and their general inability to reliably estimate the relative importance of genetic factors in determining within-population variation in thrombosis risk. Family-based studies eliminate these problems, although their costs tend to be greater.

The high additive genetic heritability that we estimated suggests that whole-genome approaches to localizing and characterizing QTLs that underlie thrombosis susceptibility will be feasible. The magnitude of the additive genetic heritability is greater than or equal to that seen in other common complex diseases such as type II diabetes (Duggirala et al. 1999*a*), gallbladder disease (Duggirala et al. 1999*b*), alcoholism (Williams et al. 1999*a*), and obesity (Comuzzie et al. 1997), whose contributing QTLs are currently being pursued through genome scans.

This is also the first study that attempts to decompose the phenotypic correlations between quantitative physiological risk factors and thrombosis into genetic and environmental components. Evidence for strong genetic correlations between FVIII, vWF, APCR, FIX, FXI, homocysteine, t-PA, and thrombosis indicate that there are sets of genes that jointly influence both disease risk and quantitative physiological variation. The detection of genetic effects that act jointly on both quantitative risk factors and disease liability is critically important for subsequent genetic analyses. When evidence of pleiotropy is detected, the correlational structure between the quantitative phenotypes and risk of thrombosis can be exploited to improve the power of joint linkage anal-

yses to detect QTLs contributing to thrombotic risk (Almasy et al. 1997).

Most of our observed phenotypic correlations are consistent with known epidemiological results. For example, there is previous evidence for a positive relationship between both vWF and FVIII levels and risk of venous (Koster et al. 1995) and arterial thrombosis (Folsom et al. 1997). High plasma homocysteine levels have been associated with deep-vein thrombosis (den Heijer et al. 1996) and with arterial thrombosis (Nygard et al. 1997). The quantitative measure of APCR is correlated with risk of venous thrombosis, even when the Factor V Leiden polymorphism is taken into account (De Visser et al. 1999). Similarly, levels of FXII (Kohler et al. 1998) and t-PA (Ridker et al. 1993; Carter et al. 1998) have been correlated with arterial thrombosis. Evidence regarding the association of FVII levels with thrombosis has been equivocal (Doggen et al. 1998; Iacoviello et al. 1998). Very recently, results from the LETS study have implicated high plasma levels of factor IX (Vlieg et al. 2000) and factor XI (Meijers et al. 2000) as risk factors for venous thrombosis.

The unique aspects of our correlational analyses lie in the ability to disentangle genetic and environmental sources of correlation. This technique allows us, for the first time, to conclude that most of the phenotypic correlations between thrombosis susceptibility and the quantitative physiological measures are due to pleiotropic effects of genes. There is little evidence that environmental effects induce much of the observed phenotypic correlations. In the two cases where we did observe significant environmental correlations, they were opposite in sign to the genetic correlations. Other investigators have reported similar results from bivariate genetic analyses of a wide variety of traits (e.g., Comuzzie et al. 1996; Brooks 2000; Mahaney et al. 2000; Stern et al. 2000). One interpretation of the difference in sign is that the genetic and environmental sources of variation on these traits act through different physiological mechanisms.

In this study, we have chosen a broad definition of thrombosis that includes both venous and arterial forms. Our justification for this is both empirical and theoretical. Pooling two genetically heterogeneous traits would decrease the genetic signal-to-noise ratio of the composite trait. However, our heritability analyses provided no evidence for such a depression in genetic signal, indicating that there must exist substantial overlap in the genetic determinants of venous and arterial forms of thrombosis. Similarly, the bivariate analysis of venous and arterial thrombosis yielded a genetic correlation not significantly different from 1 and suggests that many of the same genes are involved in the pathogenesis of venous and arterial events. Additionally, there is epidemiological evidence that similar pathways are involved in venous and arterial thrombosis, as evidenced by the correlation between critical risk factors (such as homocysteine, vWF, and FVIII) and both venous and arterial thrombosis. Although unique local environmental factors can separately influence thrombogenesis in veins and arteries, the evidence suggests that much of the underlying process is driven by a common set of genes. Pooling of these two categories of thrombosis clearly improved the power of the present study. However, even if we disaggregate these components and analyze only venous thrombosis, our results are effectively unchanged (data not shown) except for predictable alterations in observed significance values resulting from the decreased overall prevalence of disease.

Finally, these results provide strong support for using genome scans to localize and evaluate the specific QTLs involved in thrombosis susceptibility. We hope to use the information on the genetic correlations between thrombosis and quantitative phenotypes obtained in this study to maximize our potential for mapping the responsible QTLs in a genome scan currently under way.

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