Major Genetic Susceptibility for Venous Thromboembolism in Men: A Study of Danish Twins

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Background. Although several genetic determinants (mutations or polymorphisms) have been associated with increased risk of venous thromboembolism, the overall influence of genetic factors on this disease is unknown.

Methods. We linked the Danish Twin Registry, which includes twins born 1870–1953, with the Danish National Registry of Patients, comprising all hospitalizations in Denmark since 1977. We then determined the risk of venous thromboembolism as determined from discharge diagnosis.

Results. We identified 26,982 twins who were alive on 1 January 1977, and computed measures of familial and genetic association of venous thrombotic disorders. Individuals were classified according to zygosity and hospitalization with venous thromboembolism. Since 1977, 678 twins were hospitalized with an episode of venous thromboembolism. Of these, only 545 pairs (281 male pairs and 264 female pairs) were alive in 1977. For men, the concordance rates for mono- and dizygotic twin pairs, respectively, were 0.22 (95% confidence interval = 0.14 to 0.30) and 0.08 (0.04–0.12). The odds ratio (interpreted as the relative risk of venous thromboembolism for one twin, given venous thromboembolism in the partner twin) was 13.5 (7.3–24.8) among monozygotic twins and 3.8 (1.8–8.3) among dizygotic twins. The respective correlations for venous thromboembolism were 0.55 (0.38–0.70) and 0.26 (0.09–0.42). The proportion of the variance attributable to genetic effects on venous thromboembolism in males was 55% (39%–68%). The remaining variation could be attributed to men's nonfamilial environments. In contrast, for women there was no intra–twin pair similarity for venous thromboembolism.

Conclusions. We found differences in genetic susceptibility to venous thromboembolism between the sexes, with genetic factors playing a substantially stronger role in males than in females.

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Venous thromboembolism is an important public health problem that affects approximately one per 1000 individuals per year among whites.[1,2] Risk factors for venous thromboembolism include recent surgery, cancer, pregnancy, trauma, immobilization, obesity and female hormones.[1] During the last decade, genetic factors that contribute to an increased risk of venous thromboembolism have been discovered. Most of these factors are mutations or polymorphisms in genes coding for coagulation proteins. Thus, in 1965, Egeberg[3]described a pedigree in which the family suffered from venous thromboembolism caused by antithrombin deficiency. Similar disorders have been described for proteins C[4] and S.[5] In 1993 Dahlbäck *et al.*[6] described a family with thrombophilia characterized by resistance to activated protein C, caused by a mutation in coagulation factor V (factor V Leiden).[7] In 1996 Poort *et al.*[8] discovered a procoagulant mutation in coagulation factor II (factor II 20210G>A). Observational studies have shown that these mutations are risk factors for venous thromboembolism.[6,9–14]

We used population-based data from the Danish Twin Registry and the Danish National Registry of Pa-

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tients to estimate the overall contribution of genetic factors to the causation of venous thromboembolism.

Methods

Study Design

The Danish Twin Registry

The Danish Twin Registry is a nationwide and population-based registry established in 1954. It initially included twins born between 1870 and 1930 who had survived to age 6 years.[15] Birth cohorts from 1931 through 1952 were recently added to the registry, which now holds data on more than 32,000 twin pairs born between 1870 and 1953. We included only same-sex twin pairs in the present study. Zygosity was established through a questionnaire on the degree of similarity between twins in a pair. The zygosity classification has been evaluated by comparison with blood group determinants, and the proportion of misclassification was less than 5%.[16]

The Danish National Registry of Patients

The Danish National Registry of Patients was established in 1977, and includes information regarding 99.4% of all discharges from nonpsychiatric hospitals in Denmark. Recorded information includes the Civil Personal Registration number (CPR), the dates of admission and discharge, the surgical procedures performed and up to 20 discharge diagnoses, classified according to the Danish version of the International Classification of Disease (ICD). We obtained data for hospitalization because of venous thromboembolism. Pulmonary embolism and venous thrombosis were coded as 450.00–450.99 and 451.00–451.99, respectively, in ICD-8, and I26.0-I26.9 and I80.0-I80.9 in ICD-10 (used from 1994 onward). Using the 10-digit CPR number, introduced in 1968 and unique to every Danish citizen, we linked information between the two registries, obtaining hospitalization records for Danish twins for the period 1977 through 1998.

Statistical Analysis

Analyses of Twin Similarity

We assessed the similarity of mono- and dizygotic twins using probandwise concordance rates, odds ratios (ORs) and correlations for venous thromboembolism. The classic twin-study methodology is based on the fact that monozygotic twins have identical genotypes, whereas dizygotic twins share, on average, half of their genes and thus are no more genetically related than ordinary siblings. A greater phenotypic similarity in monozygotic than in dizygotic twins is to be expected if there is a substantial genetic component in the etiology of the disease.

Concordance Rates and Risk Estimates

The probandwise concordance rate is defined as the proportion of affected twin partners of probands. It reflects the probability of one twin's having a disease, given that the partner twin is affected. Thus, it is directly comparable with risk rates reported for other relatives.[17] We computed the confidence interval using the standard error for a proportion.

The odds ratios use the additional information available from concordant nondiseased twin pairs, and can be interpreted as the relative increase in risk of venous thromboembolism for one twin, given the presence *vs* the absence of venous thromboembolism in the partner twin.[18] For the confidence interval, we used standard test–based computations, except for the OR, which was based on a zero cell, for which we applied a method by Cornfield.[19]

Correlation

We estimated the correlations for venous thromboembolism (expressed as correlations attributable to a dichotomous outcome) using a multifactorial threshold model[20] and the Mx software package.[21] Likelihood-based confidence intervals were estimated by structural equation modelling, as described in detail elsewhere.[22] This assumes an underlying normally distributed liability (susceptibility) to a disease because of genetic and environmental factors. The manifestation of a disease is established when an individual exceeds the threshold of affliction on the liability distribution, and the impact of genetic and environmental effects are reflected in the similarity of the other twin's liability to the disease.[22]

Heritability

According to standard biometric practice, we assumed no epistasis (gene-gene interaction), no gene-environment interaction or correlation and no assortative mating. The phenotypic variance can then be separated into four variance components: variance attributable to additive genetic effects (A), genetic dominance (D), shared (family) environment (C), and nonshared (individual-specific) environment (E).[22] Only nonshared environments contribute to dissimilarity within monozygotic twin pairs because of their genetic identity, whereas the effects of additive genetic factors and genetic dominance may also contribute to dissimilarity within dizygotic pairs, who share, on average, half of the additive and one-quarter of the dominant genetic factors (Figure 1). The method for selecting the best model followed standard procedures (structural-equation analyses).[22]

Because the effects of genetic dominance (D) and shared environment (C) are completely confounded in the classical study of twins reared together, it is not possible to estimate all of the parameters simultaneously in a single model. Thus, we fitted five models (ACE,

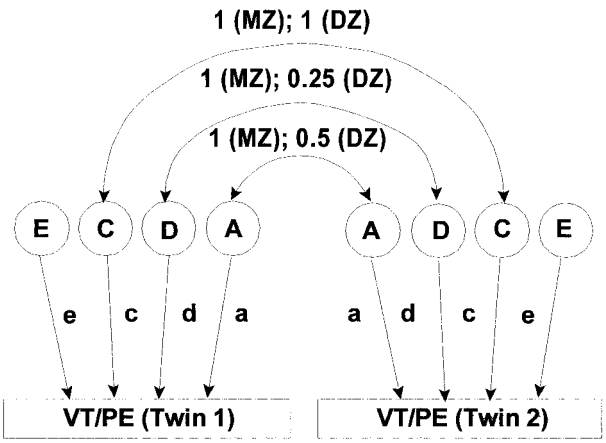

FIGURE 1. The phenotypic variance can be separated into four variance ocmponents: variance attributable to additive genetic effects (A), genetic dominance (D), shared (family) environment (C), and nonshared (individual-specific) environment (E). Details are provided in the text. MZ = monozygotic; DZ = dizygotic; VT = venous thrombosis; PE = pulmonary embolism.

ADE, AE, CE and E models) to the data summarized in Table 2. The best model is considered to be one that fits the data well (by a chi-square goodness of fit test) and is the most parsimonious (*ie*, none of the parameters in the model can be deleted without a substantial increase in the chi-square value). For comparison of non-nested models we used the Akaike Information Criterion (AIC; chi-square goodness of fit statistic minus twice the degree of freedom). The model with the lowest AIC represents the best balance of goodness of fit and parsimony.[23] For comparison among nested models, we used the chi-square difference test. The difference in chi-square of the models is itself distributed as a chi-square statistic, with the degrees of freedom equal to the difference in the degrees of freedom of the models being compared.

First, we carried out analyses of the different models, allowing the variance components to vary across sex. Then, we analyzed submodels of the best-fitting model, in which the variance components were constrained to be equal across sex groups, to test the effect of sex on the parameters. Finally, the heritability of liability to venous thromboembolism (*ie*, the proportion of the total phenotypic variance attributable to genetic variance) was derived from the best-fitting model.

Results

We identified 26,982 twins with known zygosity, who were alive on 1 January 1977. For 3802 twin pairs (13%), the co-twin had died or emigrated before 1 January 1977, leaving 11,590 twin pairs for the analyses of twin similarity.

Since 1977, 678 twins were hospitalized with an episode of venous thromboembolism (Table 1). Of these, only 545 pairs (281 male and 264 female pairs) were alive in 1977 (Table 2). The cumulative incidence rates of venous thromboembolism were similar in the two sexes and the two zygosity groups (ranging from 2.2% to 2.8%).

Among the men, the concordance rate for monozygotic twin pairs was 0.22 (95% confidence interval [CI] = 0.14–0.30) and for dizygotic twin pairs 0.08 (0.04–0.12) (Table 2). The odds ratio for the second twin to have venous thromboembolism if one twin had an event was 13.5 (7.3–24.8) for monozygotic twins and 3.8 (1.8–8.3) for dizygotic twins; the correlations were 0.55 (0.38–0.70) and 0.26 (0.09–0.42), respectively. In contrast, for monozygotic women the odds ratios were essentially 1.0 and there were no concordant dizygotic pairs among women.

The median age at first diagnosis of venous thromboembolism in the first twin was similar in monozygotic and dizygotic males (61.0 and 60.5 years, respectively). The time to diagnosis in the second twin was considerably shorter in monozygotic male pairs compared with dizygotic pairs (median time of 2 and 7.5 years, respectively, after the diagnosis in the first twin). Among the concordant twin pairs, five male pairs had a combination of venous thrombosis in one twin and pulmonary embolism in the other (four pairs of monozygotic twins and one pair of dizygotic twins). In the remaining concordant pairs, venous thrombosis occurred in both twins.

Structural-equation analyses showed that a model including additive genetic factors and nonfamilial environment provided the best fit to the data. The heritability (the

TABLE 1. Number of Danish Same-Sex Twins Born 1870–1952* Who Had a Diagnosis of Deep Venous Thromboembolism or Pulmonary Embolism in the Period 1977–1998

	Men		Women		
	Monozygotic (N = 4,336)	Dizygotic (N = 9,369)	Monozygotic (N = 4,468)	Dizygotic (N = 8,809)	Total (N = 26,982)
Deep Venous Thromboembolism	82	136	56	138	412
Pulmonary Embolism	39	104	41	82	266
Total number diseased	121	240	97	220	678
(% of Total)	(2.8%)	(2.6%)	(2.2%)	(2.5%)	(2.5%)

* Oldest person included was born in 1877.

TABLE 2. Probandwise Concordance Rate, Odds Ratio and Tetrachoric Correlation for Deep Venous Thromboembolism/ Pulmonary Embolism in Danish Twin Pairs*

Zygosity	Number of Pairs			Probandwise Concordance Rate (95% CI)†	Odds Ratio (95% CI)†	Tetrachoric Correlation (95% CI)†
	Disease Concordant	Disease Discordant	Non-Disease Concordant			
Monozygotic						
Men	11	78	1,857	0.22 (0.14 to 0.30)	13.5 (7.3 to 24.8)	0.55 (0.38 to 0.70)
Women	1	83	1,958	0.02 (0.00 to 0.06)	1.1 (0.6 to 2.1)	0.02 (−0.34 to 0.33)
Total	12	161	3,815	0.13 (0.08 to 0.18)	7.0 (3.9 to 12.7)	0.40 (0.25 to 0.54)
Dizygotic						
Men	8	184	3,967	0.08 (0.04 to 0.12)	3.8 (1.8 to 8.3)	0.26 (0.09 to 0.42)
Women	0	180	3,808	0.00 (0.00 to 0.06)	0.0 (0.0 to 1.8)	nc‡
Total	8	364	7,775	0.04 (0.02 to 0.06)	1.9 (0.8 to 4.3)	0.12 (−0.03 to 0.25)

* The tables includes only twin pairs in which both twin pairs were alive and living in Denmark in 1977.
† See text for method of CI computation.
‡ Not calculated because of the 0 cell.

proportion of the variance attributable to genetic effects) for venous thromboembolism for men was 55% (CI = 39%–68%) (Table 2). The remaining variation could be attributed to men's nonfamilial environments. In contrast, there was no intra–twin pair similarity of venous thromboembolism for women, and hence no detectable genetic effects: 0% (CI = 0%–18%).

Structural-equation analyses showed that a model including additive genetic effects and nonshared environment (AE) was the best-fitting model when parameter estimates were allowed to vary by sex (chi-square = 4.9; degrees of freedom [df] = 8; $P = 0.77$; AIC = −11.1). If the parameter estimates were constrained to be equal for males and females, this led to a marked increment in lack of model fit (chi-square = 21.0; df = 9; $P = 0.01$; AIC = 3.0). The chi-square difference test between the two models is highly statistical significant (chi-square difference = 16.1; df = 1). Similar results were obtained if sex differences were tested under an ACE model.

Discussion

This study of 11,590 twin pairs revealed major heritability for venous thromboembolism in males, but not for females. We used nationwide, population-based registries from a uniformly organized health care system, with a high validity of the zygosity classification of the twins and virtually no loss to follow-up. However, we did not have access to mortality data for patients with venous thromboembolism who died outside hospitals or data on hospitalizations before 1977. If a strong association exists between genetic factors and the severity of pulmonary embolism, this selection bias would probably lead to an underestimation of the risk caused by genetic susceptibilities. Some prevalent cases of venous thromboembolism surviving the first hospital admission before the study period might also be misclassified as incident cases if admitted for a recurrent episode during the study period. Venous thromboembolism may have been misclassified in 10% to 20% of the cases listed in Scandinavian Hospital Discharge Registries.[21] This lack of specificity, which is probably nondifferential between the sexes, would lead us to underestimate the similarity in venous thromboembolism occurrence within twin pairs.

We cannot completely rule out a type 1 error, but the large number of concordant pairs makes this possibility less likely.

We had no data about confounding factors other than age and sex. It is unlikely that selection bias and confounding could explain the entire difference in estimates between the sexes. Obesity and cancer are risk factors for venous thromboembolism,[24,25] and both of these conditions may have a weak genetic component.[26,27] However, the available evidence does not indicate a difference between the sexes in these risk factors.[27,28]

The sex differences found were unexpected. It is conceivable that the occurrence of venous thromboembolism in one twin could lead to more intensive diagnostic work-up in the other twin or make the other twin take further protective steps (such as discontinuation of oral contraceptive use or more intense monitoring during pregnancy) to reduce the risk for the second twin. However, such steps would presumably be nondifferential between mono- and dizygotic twins.

The exact genetic or biological mechanisms behind our findings are unclear. Several specific genetic abnormalities in the clotting system have been discovered.[29,30] There are well-known sex-linked clotting disorders, the most famous of which are hemophilia A and B, associated with deficiencies in coagulation factors VIII and IX, respectively. Hemophilias are disorders with loss of protein function, whereas gain of function (high concentrations of factors VIII or IX) has been linked to thrombophilia.[31,32] High factor VIII levels aggregate in families,[33] pointing towards a genetic origin. However, this genetic disorder is apparently not sex linked and thus cannot explain the absence of detectable genetic effects among females in our study. It is not clear why

males should exhibit high heritability for venous thrombotic disorders, whereas females show virtually none.

We conclude that there are differences in the genetic susceptibility to venous thromboembolism between the sexes, with genetic factors playing a substantially stronger role in males. Our findings suggest that other genes, or mechanisms of gene interaction, have yet to be identified, and that sex differences in venous thromboembolism may deserve closer attention.

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