Genetic variation and susceptibility to venous thrombosis : Etiology and risk assessment

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The Value Of Family History As A Risk Indicator For Venous Thrombosis

Chapter 2

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Archives of Internal Medicine 2009;169(6):610-615

ABSTRACT

Background A positive family history of venous thrombosis may reflect the presence of genetic risk factors. Once a risk factor has been identified, it is not known whether family history is of additional value in predicting an individual's risk. We studied the contribution of family history to risk of venous thrombosis conditional on known risk factors.

Methods In the MEGA Study, a population-based case-control study, we collected blood samples and information about family history and environmental triggers from 1605 patients with a first venous thrombosis and 2159 control subjects.

Results 505 (31%) Patients and 373 (17%) control subjects reported having one or more affected first-degree relatives. A positive family history increased the risk of venous thrombosis more than twofold (odds ratio [95% confidence interval], 2.2 [1.9-2.6]) and up to fourfold (3.9 [2.7-5.7]) when more than one relative was affected. Family history corresponded poorly with known genetic risk factors. Both in those with and without genetic or environmental risk factors, family history remained a risk indicator. The risk increased with the number of risk factors identified; for those with a genetic and environmental risk factor and a positive family history, the risk was about 64-fold the risk of those with no known risk factor and a negative family history.

Conclusions Family history is a risk indicator for a first venous thrombosis, regardless of the risk factors identified. In clinical practice the family history may be more useful for risk assessment than thrombophilia testing.

INTRODUCTION

A positive family history of venous thrombosis may reflect the presence of genetic risk factors in a family. Carriers of a genetic risk factor are at increased risk of a first venous thrombosis, particularly when exposed to environmental triggers. Factor V Leiden, for example, synergistically increases the risk of venous thrombosis in oral contraceptive users [22]. Since universal screening is not cost-effective [23,24], research efforts are focused on selection criteria that may be used to increase the chance of finding a genetic risk factor. Family history is an evident candidate.

Several authors have studied the value of family history as a surrogate of known genetic risk factors for venous thrombosis [25-29]. These studies have shown that the family history cannot be used to identify genetic risk factors because both positive predictive value and sensitivity are low.

Few have studied the association between family history and venous thrombosis [30,31]. In addition, it is not known whether family history is of additional value in predicting an individual's risk of venous thrombosis once a genetic risk factor is identified. We therefore estimated the relative risk of venous thrombosis when the family history is positive and studied the contribution of family history to risk in strata of known risk factors. Family history was evaluated in patients with venous thrombosis and control subjects from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study), a large population-based case-control study.

METHODS

Study Population and Data Collection

Recruitment, data collection and ascertainment of venous thrombosis events in the MEGA study were described in detail previously [20,32]. Patients had experienced a first deep vein thrombosis of the leg or pulmonary embolism between March 1, 1999 and August 31, 2004. Control subjects were partners of patients or

random population control subjects. The random control subjects were recruited by random digit dialing [33] between January 1, 2002 and December 1, 2004, and frequency matched on sex and age to the patient group. All participants completed a questionnaire on risk factors for venous thrombosis and family history. A blood sample was taken three months after discontinuation of vitamin K antagonist therapy from patients who were diagnosed until June 1, 2002 and their partners. Patients with an indication for life-long treatment with vitamin K antagonists were invited for a blood draw one year after the index date. Patients who were diagnosed from June 1, 2002, onwards and their partners received a cotton swab along with their questionnaire for collecting buccal cells; these were not included in the present study. In the random population control group, blood samples were collected throughout the entire study period and after returning the questionnaire. Overall response rates were 83% in the patient group, 82% in the partner control group and 69% in the random population control group.

Family history

Participants were asked whether parents, brothers or sisters had experienced venous thrombosis and, if so, the age at the event. Because partners of patients were recruited as control subjects, offspring was not included in the family history definition. Family history was considered positive if at least one of these first-degree relatives had experienced venous thrombosis. Within this group, participants with a strong indication of genetic predisposition were defined as having at least one first-degree relative affected before the age of 50 years, or having multiple first-degree relatives affected regardless of their age. When none of the first-degree relatives had suffered a venous thrombosis, family history was defined negative. The answer 'I don't know' was also considered negative.

Environmental triggers

Environmental triggers were surgery, injury (any self-reported injury, such as muscle ruptures or sprain), immobilization (plaster cast, extended bed rest at home for at least 4 days, hospitalization) and pregnancy or puerperium within three months prior to the index date, use of oral contraceptives or hormone replacement therapy at the index date and diagnosis of malignancy within five

years before or within six months after the index date. The index date was defined as date of diagnosis for patients and their partners, and date of completing the questionnaire for random controls.

Genetic risk factors

Genetic risk factors were the factor V Leiden mutation, the prothrombin 20210A mutation, low antithrombin levels, low protein C levels and low protein S levels. Since many mutations in the genes encoding antithrombin, protein C and protein S may cause deficiency, protein levels served as a surrogate for genetic defects. A sample was classified as "low" when the protein level was below the reference value calculated in control subjects (geometric mean minus 2 standard deviations). For protein C and protein S, the reference values were calculated excluding vitamin K antagonist users. In addition, we compared protein C levels to factor VII levels, and protein S levels to factor II levels in order to discriminate between "isolated" low protein C or S levels and overall low coagulation factor levels. We calculated the expected protein C level by linear regression of protein C on factor VII, and calculated the observed over expected ratio for protein C [34]. For protein S the observed over expected ratio was calculated by regression on factor II. The observed protein C or S level was classified as "low" when both the absolute value and the observed over expected ratio were below the reference value calculated in control subjects (geometric mean minus 2 standard deviations). Specific reference values of protein C and protein S levels were calculated for vitamin K antagonist users that were included in sensitivity analyses; the ratios to factor VII and factor II are independent of vitamin K antagonist use.

For the present analysis we selected participants who provided complete information about family history and environmental triggers and donated a blood sample. Among 3033 patients who filled in the questionnaire, 2712 (89%) provided information about family history and of 1959 (65%) patients complete information about environmental triggers and a blood sample were available. In the control group, 4317 (88%) of 4887 participants provided information about family history and of 2438 (50%) control subjects complete information about environmental triggers and a blood sample were available.

During pregnancy and oral contraceptive use protein S levels are reduced and cannot be used as an indicator of a genetic defect of protein S. We therefore excluded women who were pregnant (0 participants) or used oral contraceptives (146 patients and 259 control subjects) at the time of the blood draw. We also excluded vitamin K antagonist users (208 patients and 20 control subjects) because protein C and protein S levels cannot be easily interpreted under these circumstances. After these exclusions 1605 patients and 2159 control subjects remained in the analyses.

Laboratory Analysis

Collection and processing of blood samples, subsequent DNA isolation and genotyping of factor V Leiden and the prothrombin 20210A mutation have been described previously [19]. Measurements of antithrombin and protein C levels were performed with a chromogenic assay and factor II and VII level measurements were based on a mechanical clotting time assay. These measurements were performed on a STA-R coagulation analyzer following the instructions of the manufacturer (Diagnostica Stago, Asnières, France). Total protein S levels were measured by an enzyme-linked immunosorbent assay (ELISA, Diagnostica Stago, Asnières, France). The mean intra- and inter-assay coefficients of variation were 1.7% and 2.6%, respectively, for antithrombin, 1.4% and 3.5% for protein C, 2.7% and 4.2% for factor II, 3.4% and 4.0% for factor VII and 5.0% and 3.5% for protein S.

Statistical Analysis

Odds ratios (OR) and 95% confidence intervals (CI) were computed to estimate the relative risk of venous thrombosis associated with a positive family history. Taking the group with a negative family history as reference, ORs were calculated for having any affected first-degree relative (with the exception of offspring), having a first-degree relative affected before the age of 50 years, and having multiple affected first-degree relatives. Adjustment for age (continuous) and sex was performed by logistic regression. Subgroup analyses were performed within strata of known risk factors and within 10-year age categories. We calculated the positive predictive value and sensitivity

of family history to identify genetic risk factors. For the positive predictive value and sensitivity estimates, binomial 95% CIs were calculated using the normal approximation.

RESULTS

Table 1. Distribution of age, sex and individual risk factors among patients with venous thrombosis and control subjects

	Patients (N=1605)	Control Subjects (N=2159)
Median age (5th – 95th percentile)	50 (27-68)	51 (28-67)
Men, N (%)	772 (48%)	1150 (53%)
Type of VTE, N (%)		
DVT	949 (59%)	NA
PE	510 (32%)	NA
DVT & PE	191 (9%)	NA
Environmental Risk Factor, Any, N (%)	1086 (68%)	425 (20%)
Surgery	276 (17%)	63 (3%)
Injury	266 (17%)	141 (7%)
Immobilisation	496 (31%)	136 (6%)
Pregnancy/puerperium*	68 (4%)	21 (1%)
Oral contraceptives / HRT*	456 (29%)	108 (5%)
Malignancy	100 (6%)	48 (2%)
Genetic Risk Factor, Any, N (%)	393 (25%)	243 (11%)
Factor V Leiden mutation	246 (15%)	102 (5%)
Prothrombin 20210A mutation	73 (5%)	37 (2%)
Low antithrombin	39 (2%)	56 (3%)
Low protein C	35 (2%)	23 (1%)
Low protein S	26 (2%)	36 (2%)

NA=not applicable

* The pregnancy and hormone use risk factor groups included women only, but the percentages are of the total study group including men and women.

Median age and distributions of sex and individual risk factors among the 1605 patients and 2159 control subjects are listed in Table 1. Family history of venous thrombosis was positive for 505 (31%) patients and 373 (17%) control subjects (Table 2). The overall OR of a positive relative to a negative family history was 2.2 (95% CI, 1.9-2.6). The association was stronger when only family members with venous thrombosis before the age of 50 years were considered positive (OR, 2.7; 95% CI 2.2-3.4) or when several relatives were affected (OR, 3.9; 95% CI 2.7-5.7). The OR for venous thrombosis when having several relatives affected and at least one of them before the age of 50 was 4.4 (95% CI 2.8-6.9, not shown). The median (25^{th} to 75^{th} percentile) number of relatives, i.e. parents and siblings, that was reported in the questionnaire was 5 (3 to 7) in the patient group and 5 (3 to 6) in the control group.

Table 2. Distribution of first-degree family history among 1605 patients with venous thrombosis and 2159 control subjects

Family history	Patients, N (%)	Control Subjects, N (%)	OR (95% CI)
negative	1100 (69%)	1786 (83%)	1 [Reference]
Positive, any relative	505 (31%)	373 (17%)	2.2 (1.9 -2.6)
Positive, relative < 50	240 (15%)	144 (7%)	2.7 (2.2 -3.4)
Positive, > 1 relative	97 (6%)	40 (2%)	3.9 (2.7 -5.7)

In 150 of 505 (30%) patients with a positive family history a genetic risk factor was identified. A higher number of affected relatives and a younger age at which the relative was affected increased the chance to find a genetic risk factor, up to 36% for patients with several affected relatives (positive predictive value, Table 3). The negative predictive value, i.e. the chance that known genetic risk factors are indeed absent when the family history is negative, was 78%. This indicates that 22% of patients were thrombophilic carriers despite a negative family history. In the control group genetic risk factors were less prevalent than among patients and the positive predictive values were lower. The ROC-curve for any relative affected, which represents

the accuracy of family history to identify genetic risk factors, had an area under the curve of only 54% in patients and 53% in the control group. When we took the presence of a genetic risk factor as the starting point, a positive family history was reported by 38% of patient carriers and by 22% of control carriers (sensitivity, Table 3). Thus, the majority of thrombophilic carriers did not have affected relatives.

Table 3. Family history and prevalence of genetic risk factors in patients and control subjects

Study Group	Family history [a]	Known genetic risk factor [b] yes	no	Predictive value (95% CI)	Sensitivity (95% CI)
Patients	Negative	243	857	78% (75% - 80%)	NA
	positive, any relative	150	355	30% (26% - 34%)	38% (33% - 43%)
	positive, relative < 50	80	160	33% (27% - 39%)	20% (15% - 26%)
	positive, > 1 relative	35	62	36% (27% - 46%)	9% (4% - 14%)
Control Subjects	Negative	190	1596	89% (88% - 91%)	NA
	positive, any relative	53	320	14% (11% - 18%)	22% (17% - 27%)
	positive, relative < 50	19	125	13% (8% - 19%)	8% (2% - 14%)
	positive, > 1 relative	9	31	23% (10% - 35%)	4% (-3% - 11%)

[a] History of venous thrombosis among parents, brothers and sisters

[b] Low protein levels of antithrombin, protein C or protein S, factor V Leiden mutation, prothrombin 20210A mutation

In order to study the value of family history as a risk indicator when known risk factors have been measured, we grouped patients and control subjects according to type of risk factor identified: none, environmental, genetic or both (Table 4). In all strata, patients more frequently reported to have affected relatives than control subjects. So, family history is a risk indicator regardless of the presence of known risk factors.

The relative risk associated with a positive family history was of similar magnitude as the risk associated with a genetic risk factor. In the absence of environmental triggers the ORs were 2.5 for family history and 2.3 for a

genetic risk factor. In the presence of environmental triggers the ORs were 16.4 for family history and 21.2 for a genetic risk factor. The OR increased with the number of risk factors identified; for those with a combination of any genetic and acquired risk factor the risk was about 60-fold the risk of those with no known risk factor and a negative family history.

To rule out that the higher prevalence of positive family histories in patients with genetic risk factors was the result of specific combinations or the number of genetic risk factors, we stratified this group by the specific genetic risk factors. In the group that carried factor V Leiden but no other genetic risk factor (40 patients and 22 control subjects), a positive family history further increased the risk of venous thrombosis; factor V Leiden carriers with a positive family history had a 2.9 fold (95% CI 1.5-5.7) higher risk than factor V Leiden carriers with a negative family history. When an affected relative was younger than 50 years, this OR was 5.4 (95% CI 2.0-14.6) and when at least two relatives were affected 17.8 (95% CI 2.2-143.1). The other strata of specific genetic risk factors included fewer patients and control subjects thereby precluding meaningful analysis.

Genetic risk factors might play the most prominent role at young age, when environmental triggers are less prevalent. We therefore calculated ORs for family history per 10-year age category. Family history was associated with the risk of venous thrombosis in all age groups. The relative risk slightly decreased with age; the ORs (95% CIs) for any relative affected were 3.2 (1.7-6.0) at age 18-29 years, 2.4 (1.6-3.6) at age 30-39 years, 2.1 (1.5-2.8) at age 40-49 years, 2.1 (1.6-2.8) at age 50-59 years and 2.2 (1.6-3.1) at age 60-69 years. Because thrombotic events in a family accumulate during life and the risk of venous thrombosis increases with age, we further studied whether age could have confounded our results. Adjustment for age did not change any of the estimates. We also adjusted for sex to assess the impact of possible associations between oral contraceptive use and family history, but again none of the estimates changed.

Table 4. Family history in strata of known risk factors.

Risk factor	Family history [a]	Patients	Control Subjects	OR (95% CI) [b]	OR (95% CI) [c]
No known risk factor					
	negative	261 (67%)	1286 (84%)	1 [Reference]	1 [Reference]
	positive, any relative	128 (33%)	252 (16%)	2.5 (1.9 - 3.2)	2.5 (1.9 - 3.2)
	positive, < 50 years	53 (14%)	98 (6%)	2.7(1.9 -3.8)	2.7 (1.9 - 3.8)
	positive, multiple relatives	23 (6%)	27 (2%)	4.2 (2.4 - 7.4)	4.2 (2.4 - 7.4)
Environmental factor only [d]					
	negative	596 (72%)	310 (82%)	1 [Reference]	9.5 (7.8 - 11.5)
	positive, any relative	227 (28%)	68 (18%)	1.7 (1.3 - 2.4)	16.4 (12.2 - 22.2)
	positive, < 50 years	107 (13%)	27 (7%)	2.1 (1.3 - 3.2)	19.5 (12.5 - 30.4)
	positive, multiple relatives	39 (5%)	4 (1%)	5.1 (1.8 - 14.3)	48.0 (17.0 - 135.6)
Genetic factor only [e]					
	negative	71 (55%)	150 (77%)	1 [Reference]	2.3 (1.7 - 3.2)
	positive, any relative	59 (45%)	46 (23%)	2.7 (1.7 - 4.4)	6.3 (4.2 - 9.5)
	positive, < 50 years	33 (25%)	15 (8%)	4.6 (2.4 - 9.1)	10.8 (5.8 - 20.2)
	positive, multiple relatives	14 (11%)	6 (3%)	4.9 (1.8 - 13.4)	11.5 (4.4 - 30.2)
Environmental and genetic factor					
	negative	172 (65%)	40 (85%)	1 [Reference]	21.2 (14.7 - 30.6)
	positive, any relative	91 (35%)	7 (15%)	3.0 (1.3 - 7.0)	64.1 (29.4 - 139.8)
	positive, < 50 years	47 (18%)	4 (9%)	2.7 (0.9 - 8.0)	57.9 (20.7 - 162.1)
	positive, multiple relatives	21 (8%)	3 (6%)	1.6 (0.5 - 5.7)	34.5 (10.2 - 116.5)

[a] History of venous thrombosis among parents, brothers and sisters

[b] Odds ratio per stratum of type of risk factors identified

[c] Odds ratio relative to the group with no known risk factor and a negative family history

[d] surgery, injury, immobilisation and pregnancy or puerperium within 3 months preceding the index date, use of oral contraceptives or hormone replacement therapy at the index date and diagnosis of malignancy within 5 years before or within six months after the index date

[e] Low protein levels of antithrombin, protein C or protein S, factor V Leiden mutation, prothrombin 20210A mutation.

Relatives for whom the answer to the question about family history was 'I don't know' were assumed to be negative. Among patients, 238 of 1605 (15%) had at least one relative with unknown venous thrombosis history while other relatives were negative (i.e. family history assumed negative), among controls 307 of 2159 (14%) answered 'I don't know' for at least one relative. Excluding these participants from the analysis led to slightly higher risk estimates for the family history.

All analyses were repeated including vitamin K antagonist users and oral contraceptive users. Including these users influenced the family history distributions by only a few percent.

COMMENT

In a large population-based case-control study we showed that a positive family history increased the risk of venous thrombosis more than twofold, regardless of the risk factors precipitating the thrombosis. A young age of the affected relative and in particular the number of affected relatives more strongly indicated a predisposition to develop venous thrombosis.

Family history and known genetic risk factors were poorly associated, as observed previously [25-27,29,35]. Both the positive predictive value and sensitivity of family history as a test for genetic risk factors were low, with ROC-curves hardly different from a random distribution. The poor predictive value either implies the existence of unknown genetic risk factors or clustering through household effects.

Patients more frequently had a positive family history than control subjects, even when known risk factors were similar. This indicates that an unwknown, probably genetic factor has caused their disease in concert with the risk factor identified. These findings suggest that most genetic risk factors have low penetrance. Only when additional risk factors are present, venous thrombosis

will develop [6,36]. The search for novel genetic risk factors should not be limited to patients without known thrombophilia, since genetic factors that interact with already known genetic risk factors might then not be found. As most carriers of a single genetic risk factor have a negative family history, the sensitivity of family history to identify a single genetic risk factor is low.
We selected low levels of antithrombin, protein C and protein S, the factor V Leiden mutation and the prothrombin 20210A mutation as genetic risk factors. These are clear and frequent genetic risk factors for venous thrombosis. Inclusion of more genetic risk factors will increase the positive predictive value at the cost of the negative predictive value, while sensitivity may remain low. More important is that our study confirms that venous thrombosis is a multi-gene disorder. Family history will be a better surrogate for multiple genetic risk factors, including those yet unknown, than for single defects.
Relatives generally underreport disease in their family [37-41]. We believe that also in our study family history may have been underreported. It does, however, correspond to clinical practice where physicians rely on the family information given by their patient and confirmation of all relatives' disease status is not feasible. Alternatively, we might have overestimated the prevalence of positive family histories because individuals might be more prone to participate in a study when their family history is positive. As selection is most likely in the control group, we might have underestimated the effect of family history.

Antithrombin, protein C and protein S levels were determined from one blood draw. In a clinical setting, low protein levels are confirmed by a second measurement before a patient is diagnosed as deficient. A previous study among patients with venous thrombosis and control subjects [42] reported that 5 of 20 (25%) patients who initially had antithrombin levels below the lower limit of normal were low at a second measurement. Confirmation of low protein C levels occurred in 15 of 22 (68%) patients and confirmation of low protein S levels in 5 of 8 (63%) patients. Confirmation occurred less frequently in control subjects. We acknowledge that the number of individuals with truly low levels of antithrombin, protein C and protein S will be lower than presented here.

We studied whether family history is of additional value in predicting an individual's risk of venous thrombosis once a genetic risk factor has been identified. We could also reverse the question and ask whether genetic testing provides additional prognostic value once the family history has been determined. This could guide decisions on starting oral contraceptive use or taking preventive measures during immobilization. Table 4 shows that environmental risk factors together with a positive family history strongly increase the risk of venous thrombosis. In the absence of a known genetic risk factor the risk is already increased more than 15-fold. Genetic testing to identify additional risk would then not seem useful. Moreover, the positive family history could well reflect unknown genetic risk factors. When the family history is negative, an environmental risk factor would increase the risk about 10-fold to 20-fold, depending on the identification of a genetic risk factor. Given the low chance of finding a genetic risk factor when the family history is negative, genetic testing does not seem to be cost effective in this situation.

It is important to note that the results from the current study apply to the risk of a first venous thrombosis, and may not be applicable to risk of recurrent venous thrombosis. In fact, previous studies have shown that neither genetic risk factors nor family history are predictive for recurrent venous thrombosis [43,44].

We conclude that family history is a risk indicator for a first venous thrombosis, even when a genetic risk factor has been identified. In clinical practice the family history may be more useful for risk assessment than thrombophilia tests. A positive family history represents increased susceptibility on top of the risk due to known genetic and environmental factors. This additional risk is due to unknown or unmeasured risk factors.

ACKNOWLEDGMENTS

Ms. I.D. Bezemer and Drs F.R. Rosendaal and C.J.M. Doggen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. We thank the directors of the Anticoagulation Clinics of Amersfoort (M.H.H. Kramer, MD), Amsterdam (M. Remkes, MD), The Hague (E. van Meegen, MD), Rotterdam (A.A.H. Kasbergen, MD), and Utrecht (J. de Vries-Goldschmeding, MD) who made the recruitment of patients possible. The interviewers (J.C.M. van den Berg, B. Berbee, S. van der Leden, M. Roosen, and E.C. Willems of Brilman) performed the blood draws. We also thank I. de Jonge, MSc, R. Roelofsen, MSc, M. Streevelaar, L.M.J. Timmers, MSc, and J.J. Schreijer for their secretarial and administrative support and data management. The fellows J.W. Blom, MD, A. van Hylckama Vlieg, PhD, E.R. Pomp, MSc, L.W. Tick, MD, and K.J. van Stralen, MSc took part in every step of the data collection. C.J.M. van Dijk, R. van Eck, J. van der Meijden, P.J. Noordijk, and T. Visser performed the laboratory measurements. H.L. Vos supervised the technical aspects of DNA analysis. We express our gratitude to all individuals who participated in the MEGA study. This research was supported by the Netherlands Heart Foundation (NHS 98.113), the Dutch Cancer Foundation (RUL 99/1992) and the Netherlands Organisation for Scientific Research (912-03-033| 2003). The funding organizations did not play a role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript.