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ORIGINAL ARTICLE

Influence of the factor II G20210A variant or the factor V G1691A mutation on symptomatic recurrent venous thromboembolism in children: an international multicenter cohort study

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Summary. *Objective:* To determine the relative importance of the factor (F) II G20210A or FV G1691A mutations as risk factors or predictors for fatal/non-fatal recurrent venous thromboembolism (VTE) in children. *Methods:* In the present cohort, the rate of VTE recurrence and the time to recurrence in relation to FII, FV, age, and sex was determined in consecutively enrolled patients with VTE aged newborn to ≤18 years carrying the FII (n = 64) or FV (n = 194) mutation. 158 children with VTE without thrombophilia served as controls. Patients were followed for a median of 58 months. Data were pooled across participating sites to increase power and to enhance the generalizability of the data. Incidence rates were given as events per 1000 person-years. *Results:* Of the 416 children enrolled, 44 had recurrent VTE at a median of 12 months following VTE onset. The overall incidence rate of recurrence was 19.8, 57.9 in patients with the FII variant, 17.9 for FV carriers, and 11.8 in the control cohort. When comparing FII patients, FV children and the control cohort multivariate analysis (Cox regression) adjusted for age and sex showed that the FII variant (hazard ratio 2.6; 95% confidence interval 1.1–5.9) influenced the hazard for recurrent VTE. *Conclusions:* Based on multivariate analysis, the presence of the FII variant was associated with an increased risk of VTE recurrence.

Keywords: children, factor II G20210A, factor V G1691A, recurrence, venous thromboembolism.

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Introduction

The incidence of venous thromboembolism (VTE) in children is 0.07–0.14 per 10 000 children per year, 5.3 per 10 000 hospital admissions, and 24 per 10 000 admissions of neonates to neonatal intensive care units [1–6]. VTE in children often occurs as a complication of a severe underlying medical condition such as sepsis, cancer, congenital heart disease, and as a result of therapeutic interventions especially the presence of central venous lines [1–12]. Inherited thrombophilias (i.e. antithrombin-, protein C-, and protein S-deficiency) and the mutations of coagulation factor (F) V G1691A and FII G20210A have been established as risk factors for VTE in adults [13–17], and have been described as additional risk factors in children [7,18–36].

Pediatric VTE can lead to the development of post-thrombotic syndrome, a potentially severe complication in more than one-third of patients [33]. Furthermore, cumulative recurrence rates following a first event have been found to be 3% in neonates and up to 8% in older children over follow-up periods between one and 10 years [5,6,12,29,35,36]. The importance of the FII and FV mutations in determining the risk of a second VTE is unknown. We investigated the importance of the FII and FV mutations for recurrent VTE in pediatric patients who possess one or both of these traits in comparison to children with no acquired or inherited

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thrombophilia. The objectives of the present study were to evaluate, among these patients: (i) the incidence of fatal and non-fatal recurrence; (ii) the time to recurrence; (iii) the predictive role, if any, of these traits for recurrent VTE.

Patients and methods

Ethics

The present multicenter cohort study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki and was approved by the medical ethics committee of the University of Münster, Germany and participating institutions in Israel and the United States. Written informed consent was given, as required.

Study design and study groups

The study design was a multicenter cohort study to assess the rate of VTE recurrence following a first VTE. Data were pooled across participating sites to enhance the generalizability of the data and to increase the power to detect predictive factors for recurrent VTE, given historical evidence for the relative infrequency of such events in children.

From January 1994 to December 2006, 416 pediatric patients with VTE aged newborn to ≤18 years carrying the FII variant (n = 64), the FV mutation (n = 194), or no acquired or inherited thrombophilia (n = 158) were consecutively enrolled via pediatric VTE cohorts in Germany, Israel, and the United States, and pooled for the present analysis. The core protocol was developed by the German collaborative group and was adopted by centers in Israel and the United States [3,19,26,27,35,36]. The development of recurrent VTE and the time to recurrence were ascertained at follow-up in survivors (end of follow-up: December 2007). Preterm infants, patients older than 18 years at onset, and children with significant additional thrombophilias (specifically, antiphospholipid antibody syndrome or deficiencies of protein C, protein S, or antithrombin, elevated lipoprotein (a), FVIIIC, or homocysteine) were excluded. In addition, children with a first ischemic stroke (i.e. ischemic stroke without multifocal pattern of cerebral infarction, ischemic stroke associated with any stenosing cerebral vasculopathy, vasculitis, moyamoya or dissection) were excluded. Apart from the exclusion criteria, no further children presenting with newly diagnosed VTE within the catchment areas were excluded [for details, see 3,19,26,27,35,36]. First VTE was diagnosed locally by standard imaging methods [i.e. for deep venous thrombosis: compression sonography with Doppler, venography, computed tomography (CT), magnetic resonance imaging (MRI); for pulmonary embolism: spiral CT, ventilation-perfusion lung scans] performed in the acute phase of a new vascular accident showing fresh thrombotic material within the lumen of a vein (or in the case of pulmonary embolism, within the pulmonary arterial tree).

As previously described [27,35,36], bacterial or viral infections, trauma, surgery, dehydration, immobilization (bed rest > four days) or obesity [body mass index (BMI) > 90th age-dependent percentiles], central venous lines, solid tumors, leukemia and lymphomas, anemia, autoimmune diseases or other chronic inflammatory conditions, renal diseases, metabolic disorders, birth asphyxia, and cardiac malformations were predefined as predisposing clinical conditions. In addition, drugs such as corticosteroids and Escherichia coli asparaginase, the use of sympathomimetics, coagulation factor concentrates, or oral contraceptives and nicotine use were classified as predisposing risk factors. VTE events occurring in the absence of one of the aforementioned predisposing clinical conditions or risk factors were classified as 'spontaneous' [27,36].

As a possible outcome predictor variable, venous patency was evaluated by the same radiologic team using the above-mentioned standard imaging methods three to six months after acute VTE onset and was classified by experienced pediatric radiologists in Germany, Israel, and the United States in the following manner: (i) 'thrombus resolution' if no clot was present in the previously affected vessel; (ii) 'thrombus reduction' if the clot was persistent but had decreased in extent or occlusiveness; (iii) 'thrombus stability' if the thrombus remained of similar extent and occlusiveness; (iv) 'thrombus progression' if the clot increased in extent or occlusiveness [27,35,36]. The latter three categories constituted a larger class of persistent thrombosis, in contrast to the category of thrombus resolution. Recurrent VTEs were confirmed by experienced radiologists who were unaware of the laboratory test results, when imaging performed in the acute phase of a new event showed fresh thrombotic material within a lumen of the vein (i.e. a new intraluminal filling defect compared with the previous radiologic imaging studies) [27,35]. Imaging results in children with and without recurrent VTE were independently confirmed by a second radiologist.

Anticoagulation

According to published pediatric treatment guidelines and the current standard of care, patients were treated on an individual basis with an acute regimen consisting of low molecular weight heparin (LMWH) or unfractionated heparin (UFH), followed by subacute therapy with LMWH or vitamin K antagonists [37]. Currently, there are no evidenced-based recommendations available with respect to secondary VTE prevention in children.

Follow-up

Routine diagnostics for cohort subjects included face-to-face physical examination by an experienced physician and standard imaging methods, at first thrombotic onset as well as at 8–12 weeks and (for persistent thrombosis) six months post-diagnosis. Following discontinuation of anticoagulation, asymptomatic pediatric patients were followed up every three to six months for the first year and at minimum yearly thereafter. In children with clinically suspected VTE recurrence, imaging was additionally performed at symptomatic presentation.

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Laboratory analyses

Laboratory analyses were uniform across the participating study centers and consisted of a comprehensive thrombophilia evaluation according to standardized recommendations [14,15,26,27]. This evaluation included testing for the FII variant and the FV mutation, antiphospholipid antibodies (including the lupus anticoagulant at minimum), and levels of antithrombin (activity assay), protein C (activity assay), and protein S (activity assay/free protein S-antigen), lipoprotein (a), FVIII and fasting homocysteine in all cases. For children enrolled in the local cohort studies before 1996, FII genotyping and lipoprotein (a) measurement was completed during routine follow-up visits starting in 1997. The diagnosis of a clotting disorder for conditions assessed by plasma-based assays was made only if the specific level was outside the reference range. Apart from the classification based on age-dependent reference ranges and confirmation of a suspected protein-based prothrombotic defect in a second plasma sample (three to six months later, not on vitamin K antagonists), the presence of a hemostatic defect in at least one first degree relative or the identification of a causative gene mutation were considered confirmatory for its hereditary nature.

Statistics

Clinical and laboratory characteristics of continuous data were presented as median (minimum–maximum, min–max) and inter-group differences in distributions were evaluated by non-parametric statistics, using the Wilcoxon/Mann–Whitney *U*-test. For categorical data, frequency distributions were compared between groups using the chi-square test or Fisher's exact test, as appropriate. For the estimation of incidence of recurrent VTE in children with FII or FV mutations or no thrombophilia, the number of events, person-time (PT) in years (y), rates and 95% confidence intervals (95% CIs) were calculated: for incidence rates, the numerator consisted of the number of patients who developed recurrent VTE during the observation period and the denominator consisted of the total study population expressed in 1000 person-years (py).

Cumulative probability of recurrent thrombosis-free survival as a function of time was determined using the method of Kaplan and Meier, with censoring for death (unrelated to recurrent VTE) or loss to follow-up; homozygous FII or FV carriers or children with combined deficiencies (dual heterozygotes; FII or FV combined with any other acquired/genetic trait) were excluded. The logrank test was used to test for differences in recurrent thrombosis-free survival between groups. In order to evaluate the contribution to the risk of recurrent VTE hazard ratios (HRs) together with 95% CIs were calculated using the Cox proportional hazards model. The primary predictor variable consisted of the type of variant (FII, FV, or no thrombophilia), and adjustment variables included age at first VTE onset, and sex. Adjusted risks were expressed as HR together with 95% CIs. The degree of agreement beyond chance between first and second reader (vascular imaging) was measured with the kappa statistics (German cohort). All statistical analyses were performed using Stata 8.0 (College Station, TX, USA) and StatView 5 (SAS Institute Inc., Cary, NC, USA).

Results

Patient population

Patient selection including reasons for exclusion is shown in Fig. 1. From the 1112 consecutively diagnosed patients, all agreed to participate in the study. Out of 1112 children, 652 were excluded, mainly because they had deficiencies of natural anticoagulants, and 44 (4%) were lost to follow-up, leaving 416 for follow-up and analysis. Six of 416 patients died acutely due to thromboembolic complications (FII $n = 3$; FV $n = 3$) and 410 patients [median age of 3.9 years (male $n = 225$)] survived. Among these, 292 (71.2%) had at least one underlying medical condition at VTE onset. There were no differences between Europe, Israel, and the United States with respect to distribution of sex, underlying medical conditions, extent of initial VTE, anticoagulant type, or anticoagulation duration, distribution of FII vs. FV mutations, and proportion of patients with recurrent VTE. Patient characteristics are shown in Table 1.

Incidence (i.e. risk) of recurrent VTE

Of the 416 patients enrolled, 44 developed recurrent VTE over a person-time of 2220 y, that is, an incidence of recurrence of 19.8 (95% CI 14.3–26.7 per 1000 py), at a median (min–max) time of 12 (0.1–60) months, from the first VTE onset. Among patients suffering from recurrent VTE, 38.6% occurred within the first six months, and 72.7% within the first year following VTE onset. In patients with recurrence sex distribution, time to recurrence, health–disease status, location of recurrent VTE (defined as fresh thrombotic material within a lumen of the vein compared with the previous radiologic imaging studies), and mode and duration of anticoagulation is shown in Table 2. The incidence rate of recurrence was 57.9 (95% CI 31.7–97.3 per 1000 py) for patients with the FII variant [14 events (fatal/non-fatal)/PT 241 y], 17.9 [95% CI 10.7–28.4 per 1000 py] for carriers of the FV mutation [18 events (fatal/non-fatal)/PT 1001 y], and 11.8 [95% CI 5.9–21.2 per 1000 py] in the control cohort [12 events (non-fatal)/PT 1013 y]. Median (min–max) age at recurrence was 3.9 (0.1–18) years, 20 patients were male, and in 34 recurrence occurred after withdrawal of anticoagulation.

Time to recurrence

The cumulative recurrence-free survival in children carrying the FII variant, the FV mutation, or no thrombophilia with a maximum follow-up time of 12 years is presented in Fig. 2.

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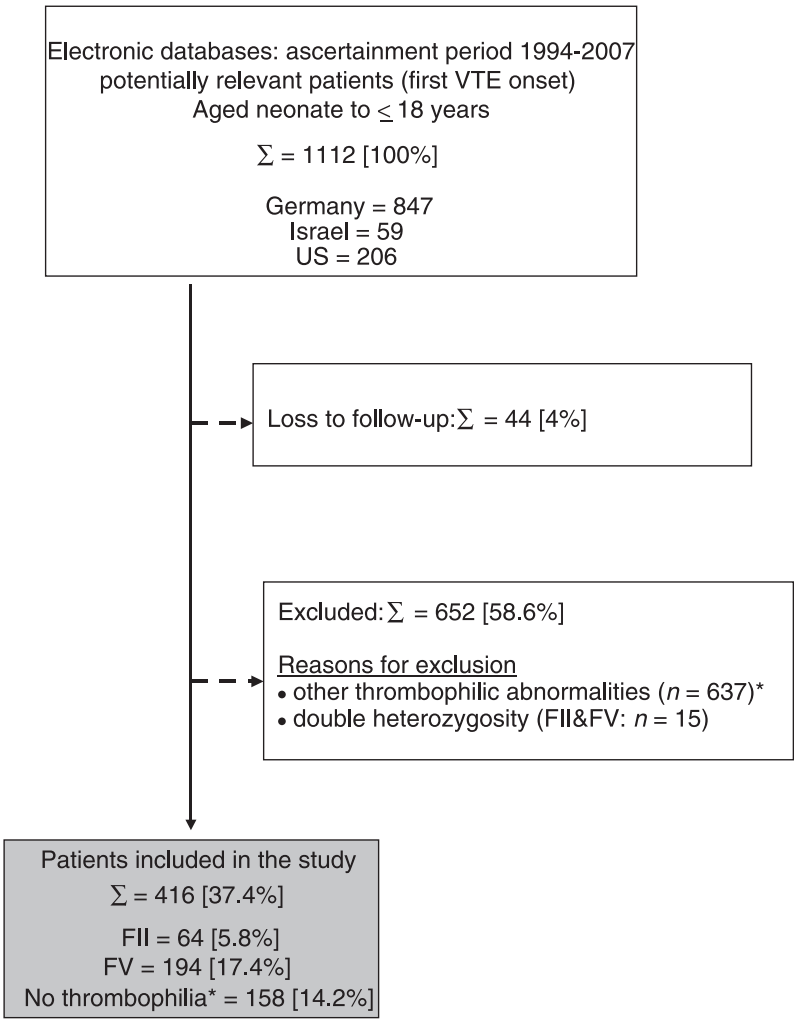

Fig. 1. A flow chart showing the results of patient selection and reasons for exclusion.

There was a significant difference between the cumulative recurrence-free survival curves, with presence of the FII variant associated with a heightened risk of recurrent VTE over time.

Multivariate analyses adjusted for age and sex show that the FII variant was associated with the risk of recurrent VTE (HR 2.6; 95% CI 1.1–5.9). The FV mutation (HR 0.8; 95% CI 0.34–1.92) did not significantly influence recurrence.

Complete data on thrombus status (resolution vs. persistence) by repeated imaging at three to six months post-diagnosis of initial VTE was available in 250 children. Agreement between first and second reader, as analyzed in the German cohort was good (kappa = 0.76). The cumulative recurrence-free survival with respect to thrombus resolution is presented in Fig. 3. Children with thrombus resolution showed a decreased risk for recurrent VTE over time when compared with subjects with persistent thrombosis.

Discussion

In this study, the overall incidence rate of a second VTE was 19.8 per 1000 py within a median period of 12 months after the initial VTE among children who carry the FII variant, the FV mutation, or no thrombophilia. The rate of adverse outcome was higher in children carrying the FII variant compared with FV carriers or children with no thrombophilia.

The overall recurrence rate reported here in children with VTE is in accordance with recently published studies on recurrent VTE in adult patients in whom the rate of any second thrombotic events ranged from 3% to 13% of patients after one year [38–40]. In pediatric cohorts, the cumulative recurrence rates reported ranged from 3% in neonates to 8% in older children (follow-up periods ranging from one to 10 years), [6,12,35,36] with a peak of 21% in children with a first idiopathic VTE observed after a median follow-up duration of two to four years [27].

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Table 1 Characteristics of patients with no thrombophilia (controls), the factor (F) II or FV mutation

	No thrombophilia	FII G20210A	FV G1691A
Acute VTE-related deaths	0/158 (0%)	3/64 (4.7%)	3/194 (1.5%)
Number of surviving children	158/158	61/64	191/194
Ethnicity			
Caucasian	150	55	175
Black	1	1	1
Middle east	2	1	4
Mixed	5	4	11
Sex			
Male, *n* (%)	95 (60.1)	28 (45.9)	102 (53.4)
Age at VTE onset, median [min–max] (years)			
Total	1.9 [0–18]	8.5 [0–18]	1.8 [0–18]
Male	2.3 [0–16.5]	6.5 [0.1–17]	1.0 [0–18]
Female	1.5 [0–18]	10.0 [0–18]	6.2 [0.1–17.5]
Health–disease status (%)			
Idiopathic/healthy	48 (30.4)	15 (24.6)	61 (31.9)
Infection	25 (15.8)	10 (16.4)	22 (11.6)
Surgery/immobilization	11 (6.7)	6 (9.8)	12 (6.3)
Central venous line (CVL)	10 (6.3)	6 (9.8)	12 (6.3)
Hematologic malignancies/CVL	10 (6.3)	3 (4.9)	20 (10.5)
Obesity	3 (1.9)	2 (3.3)	5 (2.6)
Oral contraceptives	4 (2.5)	5 (8.2)	6 (3.2)
Congenital heart disease/CVL	13 (8.2)	2 (3.3)	7 (3.7)
Asphyxia	5 (3.2)	–	5 (2.6)
Miscellaneous > $n = 3$*	29 (18.4)	12 (19.7)	41 (21.6)
VTE locations			
Calf	9 (5.7)	5 (8.2)	20 (10.5)
Proximal leg	22 (13.9)	9 (14.8)	20 (10.5)
Proximal leg and pelvis	16 (10.1)	7 (11.7)	14 (7.3)
Renal/portal	10/6 (6.3/3.8)	1/- (1.6/-)	14/3 (7.3/1.6)
Pulmonary embolism	3 (1.9)	4 (6.6)	5 (2.6)
Leg and pulmonary embolism	5 (3.2)	–	3 (1.6)
Jugular/subclavian veins	15 (9.5)	–	22 (11.3)
Intracardiac	5 (3.2)	2 (3.3)	5 (2.6)
Cerebral veins	44 (28.8)	23 (37.7)	49 (25.8)
Multiple (≥2)	23 (14.6)	10 (15.6)	36 (18.8)
Duration of anticoagulation following first VTE: median [min–max]	6 [0–100]	6 [0–84]	6 [0–96]
Duration of follow-up (months): median [min–max]	59 [12–156]	54 [12–156]	61 [12–156]

*Combined underling medical conditions: dehydration, diabetes Type 1, fetopathia diabetica, immobilization, infection, nephrotic syndrome, obesity, smoking, oral contraceptives, trauma. VTE, venous thromboembolism.

The results of this study demonstrate that children with thrombosis who are heterozygous for either the FII or FV mutation, or who did not carry an acquired or inherited thrombophilia developed an adverse outcome, which is consistent with the rate reported in large pediatric cohort studies, which did not investigate genetic traits [1,5,6]. Recurrent events in this study, however, occurred in the majority of cases after withdrawal of anticoagulant therapy. Put into context of present guidelines for antithrombotic management in adult VTE, the recommended duration of anticoagulant therapy for a first thrombotic episode is at least three months, and indefinite anticoagulation should be considered in patients with idiopathic VTE and a low bleeding risk [41]. These guidelines are typically extrapolated to children [37]; however, the benefit-to-risk ratio of prolonged use of anticoagulation in highly physically active children must be taken into consideration. Based on the fact that recurrent VTEs have occurred most often after cessation of anticoagulation, future studies of anticoagulation in children should consider a risk-stratification approach as the risk of recurrence not only differs in patients of different ages, but also in carriers of different thrombophilias. The issue of secondary thrombosis prophylaxis in situations conferring a high-risk of recurrent VTE (particularly in those found to be at higher risk in this study), such as dehydration, hematologic malignancy, an indwelling central venous lines, severe infection/inflammation, or prolonged immobilization needs to be clarified.

Limitations of the present cohort study include the non-availability of three to six months patency data in all children and the absence of central reading of acute and follow-up imaging for all patients with assessment of interobserver variability. The latter would be an important component of a prospective study in future. Shortcomings of this study further

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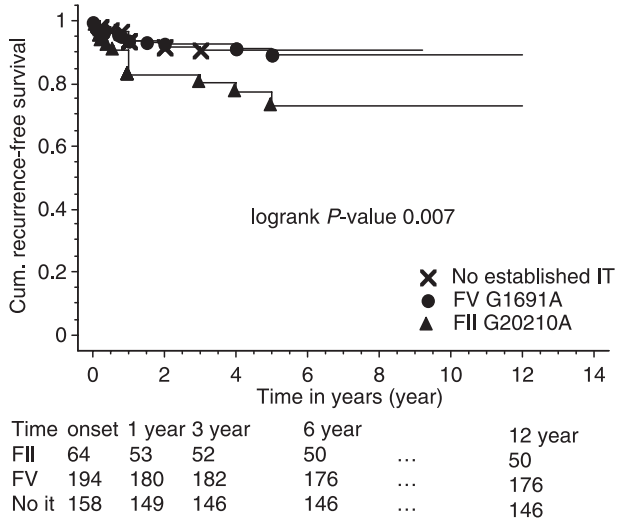
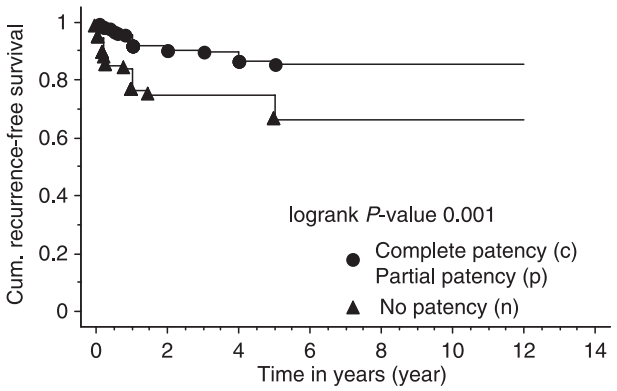
Table 2 Characteristics of factor (F) II and FV carriers with recurrent venous thromboembolism (VTE) compared with children with recurrence and no thrombophilia

	No thrombophilia	FII G20210A	FV G1691A	*P*-value
Total number of patients	158	64	194	
Number of surviving patients	158/158	61/64	191/194	0.03
Number of recurrent VTE in surviving patients, *n* (%)	12 (7.6)	11 (18.0)	15 (7.9)	0.04
Ethnicity, *n* (%)				
Caucasian	11 (91.6)	10 (91)	14 (93.3)	0.96
Black	1 (8.4)	1 (9)	1 (7.1)	
Sex				
Male, *n* (%)	7 (58.3)	4 (36.4)	9 (60.0)	0.45
Time to recurrence median [min–max] months	12 [0.2–36]	12 [0.1–60]	9 [0.1–60]	0.86
Health–disease status, *n* (%)				
Idiopathic/healthy	4 (33.3)	2 (18.1)	4 (26.6)	0.78
At least one underlying trigger	8 (66.6)	9 (81.8)	11 (78.6)	
New VTE formation at recurrence				
Proximal leg	4	4	3	0.60
Pelvis	2	2	4	
Renal/portal	–	–	2/1	
Pulmonary embolism	1	2	–	
Intracardiac	1	1	1	
Cerebral veins	3	2	4	
Jugular/subclavian veins	1	–	–	
Anticoagulation performed after first VTE onset, *n* (%)	10 (83)	10 (91)	11 (78.6)	0.54
Low molecular weight heparin	5	5	5	
Warfarin	5	4	7	
Aspirin	–	1	–	
Treatment duration following first VTE				
Median [min–max] months	6 [0–48]	6 [0–12]	5 [0–36]	0.67
Anticoagulation prior relapse, *n* (%)				
Yes	1 (8.3)	2 (18.1)	1 (7.1)	0.54
No	11 (91.7)	9 (81.8)	14 (93.3)	



Time	onset	1 year	3 year	6 year		12 year
FII	64	53	52	50	…	50
FV	194	180	182	176	…	176
No it	158	149	146	146	…	146

Fig. 2. The cumulative recurrence-free survival in children carrying no thrombophilia (IT), the factor (F) II variant or the FV mutation with a maximum follow-up time of 12 years. In addition, the number of patients at risk is included. The logrank test showed a statistically significant difference between the cumulative recurrence-free survival curves.



Time	0.5 years	3 years	6 years		12 years
Total	250	216	210	…	210
c/p	176	160	156	…	156
n	74	56	54	…	54

Fig. 3. The cumulative recurrence-free survival with respect to thrombus resolution. 176 children with data on complete thrombus resolution (circle) available showed a decreased risk for recurrent venous thromboembolism over time when compared with subjects with persistent/progressive thrombosis ($n = 74$; triangle). The logrank test showed a statistically significant difference between the curves when comparing children with and without thrombus resolution. In addition, the number of patients at risk is given.

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include the fact that children in this study were principally Caucasian, were ascertained in the majority of cases from Germany, and that the only thrombophilias studied here were the FII and FV mutation in comparison to children with no thrombophilia. Thus, the data presented here could neither be extrapolated to pediatric VTE patients of other ethnicities, nor to children with other thrombophilias, such as antithrombin-, protein C-, S-deficiency, or elevated levels of FVIII, homocysteine, and lipoprotein (a). Our finding that the overall recurrence rates are similar to those previously reported in pediatric cohorts, which did not investigate genetic traits [1,5,6], suggests that the data herein may be more generalizable. The latter, however, does not mean that children with VTE should not be screened for thrombophilia. An individual thrombophilia screening program in selected patient cohorts for which the population background suggests an association between VTE and thrombophilia of interest as 'obvious' will be meaningful.

In conclusion, compared with children with the FV mutation or no thrombophilia, children with the FII variant are at increased risk for recurrent VTE. Future prospective studies should be aimed at further delineating the risk factors for recurrent VTE in children as this could have significant implications on outcome and possibly treatment modalities.

Addendum

Along with the principal study investigators (i.e. G. Young, N. Goldenberg, and U. Nowak-Göttl who act as the guarantors), all other investigators had full access to the data (S. Becker, F. Friedrichs, C. Düring, G. Kenet, M. Manco-Johnson, and C. Scheffold) and took part in the design, execution and data analysis, and in writing the report. F. Friedrichs and U. Nowak-Göttl were responsible for the statistical calculation.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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