

Risk of recurrent venous thrombosis in children with combined prothrombotic risk factors

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After a first episode of spontaneous venous thromboembolism (VTE), the risk of recurrence persists for many years. However, comprehensive data about the risk of recurrence in pediatric patients have hitherto not been reported. Thus, this study evaluated the risk of recurrent VTE among children in relation to the presence of single or combined-inherited and/or acquired causes of thrombophilia. A total of 301 patients aged neonate to 18 years (median, 6 years) who were referred for an objectively confirmed first episode of spontaneous VTE were followed prospectively for a median time of 7 years (range, 6 months to 15 years) after with-

drawal of anticoagulation. All patients were studied for established acquired and inherited causes of thromboembolism. With reference to all 301 patients, one single prothrombotic risk factor was found in 176 subjects (58.5%), whereas combined defects were found in 20.6% (n = 62). Recurrent VTE occurred in 64 patients (21.3%) within a median time of 3.5 years (range, 7 weeks to 15 years) after withdrawal of anticoagulation, with a significantly shorter cumulative thrombosis-free survival in children carrying combined defects ($P < .0001$; chi-square, 42.2). The factor V G1691A mutation was present in the majority of patients with

recurrent VTE. Including genetic defects, gender, and acquired risk factors, multivariate analysis showed that only the presence of prothrombotic defects increases the risk of recurrent VTE (single defect: odds ratio [OR], 4.6; 95% confidence interval [CI], 2.3-9.0; $P < .0001$; combined defect: OR, 24.0; 95% CI: 5.3-108.7; $P < .0001$). As a consequence of the data presented here, it is suggested that screening for genetic risk factors be done among pediatric patients with VTE. (Blood. 2001;97:858-862)

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Introduction

Venous thromboembolism (VTE) caused by acquired factors (eg, trauma, surgery, prolonged immobilization, and intake of oral contraceptives as well as by inherited predisposing factors) is the third most common vascular disease among Caucasians.¹ Various molecular defects of different hemostatic components have been established as risk factors for thromboembolic diseases mainly in adults: deficiencies of protein C, protein S, and antithrombin, resistance to activated protein C mostly due to the factor (F) V G1691A gene mutation,²⁻⁴ and the prothrombin (PT) G20210A genotype.⁵

Patients who have had thromboembolic disease remain at risk for VTE after withdrawal of adequate treatment with heparin or coumarin derivatives.⁶ Complying with the recognition of venous thrombosis as a multifactorial disease and with the observation that coinheritance of prothrombotic factors substantially increases the risk of primary VTE,⁷⁻¹¹ there is increasing evidence that carriers of combined prothrombotic risk factors are more prone to recurrent thrombotic episodes than subjects with no or only one inherited prothrombotic defect.¹²⁻¹⁵ This issue is clinically important, because carriers of

combined defects might be candidates for long-term anticoagulant therapy if their risk of recurrent thrombosis is high in childhood and adolescence. However, because the majority of studies regarding recurrence of VTE have been performed in adult patients,¹²⁻¹⁶ only little information is available on the prothrombotic risk profile and rate of recurrence in childhood VTE. Although it has recently been shown that established clotting abnormalities (ie, deficiencies of protein C and antithrombin, the presence of the FV G1691A and the PT G20210A gene mutations as well as elevated lipoprotein (a) [Lp(a)]) are also independent risk factors of childhood venous thrombosis and childhood ischemic stroke,¹⁷⁻¹⁹ experience of recurrent VTE in pediatric patients is still limited. Thus, the objective of the present prospective study was to evaluate the prevalence of recurrent VTE in a population of consecutively recruited children with a first episode of spontaneous VTE and to investigate whether individuals carrying one or more prothrombotic risk factors have an increased risk of recurrent VTE in childhood and adolescence.

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A complete list of the members of the Childhood Thrombophilia Study Group appears in the Appendix.

Submitted June 13, 2000; accepted October 6, 2000.

Supported by grants from the Medical Faculty of the University of Münster (IMF), the Landesversicherungsanstalt Westfalen, and the Landesversicherungsanstalt Rheinprovinz.

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Patients and methods

Study period and inclusion criteria

From May 1985 to May 1999, 301 Caucasian pediatric patients (female $n = 169$, male $n = 132$) with a first spontaneous VTE (not associated with immobilization, trauma, surgery, plaster casts, leukemia, cancer, central venous lines, bacterial or viral infections, autoimmune disease, or intake of oral contraceptives) of the total group of 499 consecutively admitted children with thrombosis, aged neonate to 18 years (median age at first thrombotic onset, 6 years) (Figure 1), were enrolled in the present study. All patients were referred to the participating study centers for treatment and/or assessment of possible causes of thrombophilia.

Anticoagulation following first symptomatic VTE

At the discretion of the participating study centers, pediatric patients with a first VTE received coumarin (international normalized ratio, 2.2-3; $n = 113$) or low molecular weight heparin (once daily, 4-hour anti-Xa activity 0.3-0.6 IU/mL; $n = 187$) for 6 months.

Exclusion criteria

From the total group of 499 consecutively recruited children, 198 patients were excluded from the study if they were diagnosed with leukemia or cancer ($n = 34$), catheter-related thrombosis ($n = 22$), autoimmune disorders including primary antiphospholipid syndrome ($n = 11$) at the first thrombotic event; if they were receiving long-term anticoagulation (> 6 months; $n = 34$); or if recurrent VTE was registered during the initial 6-month anticoagulation period (coumarin group only, $n = 7$; homozygous FV A1691A gene mutation, $n = 2$; increased Lp(a), $n = 3$; without identified defect so far, $n = 2$). In addition, patients without complete diagnostic work-up ($n = 39$) or for whom parental consent was refused ($n = 29$) were not enrolled in the study. Because of nonthrombosis-related death ($n = 6$) or loss of follow-up ($n = 16$), a further 22 of the 499 children could not be included in the study.

Study end point

The end point of the study was prospectively defined as symptomatic recurrent VTE or thrombosis-associated death after withdrawal of initial prophylactic anticoagulation. Objective confirmation of VTE or VTE-related death was performed by standard imaging methods (venography, compression ultrasonography, computed tomography [CT], magnetic resonance imaging [MRI], perfusion lung scan, autopsy) carried out at first thrombotic onset, and 6 to 8 weeks and 6 months later (before withdrawal of anticoagulation). In asymptomatic pediatric patients, 2 further routine imaging controls were performed 6 and 12 months after withdrawal of anticoagulation. Venography, CT, and MRI were the imaging methods used to confirm clinically suspected recurrent VTE. Recurrent VTE in the deep

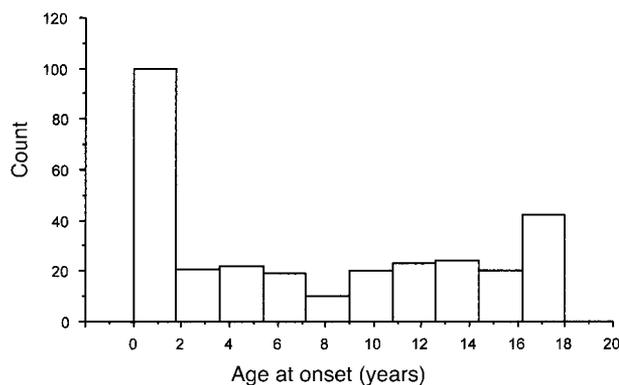


Figure 1. Age-distribution at the first thrombotic onset in pediatric patients with spontaneous venous thrombosis not associated with secondary causes of thrombosis.

veins of the leg was defined when venography performed in the acute phase of a new vascular accident showed fresh thrombotic material within a lumen of the vein (ie, a new intraluminal filling defect compared with the previous tests).

Classification of recurrent VTE

In each patient, recurrent VTE was classified as occurring spontaneously or associated with acquired risk factors predisposing to thrombosis (ie, recent immobilization, surgery, trauma, plaster casts [< 6 weeks before VTE], severe bacterial or viral infection, autoimmune disease, and/or intake of oral contraceptives [females]).

Blood sample collection

With informed parental consent, blood samples were collected after withdrawal of anticoagulation by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt, Nümbrecht, Germany) and placed immediately on melting ice. Platelet poor plasma was prepared by centrifugation at 3000g for 20 minutes at 4°C, aliquoted in polystyrene tubes, stored at -70°C , and thawed immediately before the assay procedure. The laboratory staff was unaware of whether the blood samples came from a patient with a first episode of VTE or with recurrent VTE. For genetic analysis, which was performed between 1996 and 1999 in all study patients, we obtained venous blood in EDTA-treated sample tubes (Sarstedt), from which cells were separated by centrifugation at 3000g for 15 minutes. The buffy coat layer was then removed and stored at -70°C , pending DNA extraction by a spin column procedure (Qiagen, Hilden, Germany).

Assays for genotyping

The FV G1691A and the PT G20210A genotypes were determined by polymerase chain reaction and analysis of restriction fragments as previously reported.^{4,5}

Plasma-based assays

Amidolytic protein C and antithrombin activities were measured on an ACL 300 analyzer (Instrumentation Laboratory, Munich, Germany), using chromogenic substrates (Chromogenix, Mölndal, Sweden). Free protein S antigen, total protein S, and protein C antigen were measured, using commercially available enzyme-linked immunosorbent assay kits (Stago, Asnières-sur-Seine, France).

Classification of deficiency states

A type I deficiency (antithrombin, protein C) state was diagnosed when functional plasma activity and immunological antigen concentration of a protein were below 50% of normal of the lower age-related limit. A type II deficiency (antithrombin, protein C) was diagnosed with repeatedly low functional activity levels along with normal antigen concentrations. The diagnosis of protein S deficiency was based on reduced free protein S antigen levels combined with decreased or normal total protein S antigen concentrations respectively. Lp(a) was determined with the COALIZA Lp(a) assay kit (Chromogenix). Lp(a) levels more than 30 mg/dL (< 28 kringle 4 repeats) were defined as elevated.¹⁷⁻²⁰

Ethics

The present study was performed in accordance with the ethical standards laid down in an updated version of the 1964 Declaration of Helsinki and approved by the medical ethics committees at the Johann Wolfgang Goethe-University, Frankfurt am Main, and at the Westfälische Wilhelms-University, Münster, Germany.

Statistical analysis

Statistical analysis was performed with the Stat View program (SAS Institute, Cary, NC). The probability of recurrent VTE as a function of time was determined with the Kaplan-Meier method, including the log-rank test to compare the recurrence-free survival in patients carrying one single

Table 1. Prevalence of single prothrombotic defects in childhood venous thrombosis in relation to recurrence status

Single prothrombotic defects (n = 176)	Prevalence n (%) in all patients (n = 301)	Prevalence n (%) in patients without recurrent VTE (n = 237)	Prevalence n (%) in patients with recurrent VTE (n = 64)	Prevalence (%) of recurrent VTE in carriers
FV 1691GA	88 (29.2)	71 (30.0)	17 (26.5)	17/88 (19.3)
Elevated Lp(a)	48 (14.9)	42 (17.7)	6 (9.4)	6/48 (12.5)
PT 20210GA	11 (3.7)	9 (3.8)	2 (3.1)	2/11 (18.2)
Protein C deficiency	20 (6.6)	15 (6.3)	5 (7.8)	5/20 (25.0)
Protein S deficiency	4 (1.3)	4 (1.7)	—	—
Antithrombin deficiency	5 (1.7)	4 (1.7)	1 (1.6)	1/5 (20.0)
Total	176 (58.5)	145 (61.2)	31 (48.4)	31/176 (17.6)

VTE indicates venous thromboembolism; FV, factor V; Lp(a), lipoprotein (a); PT, prothrombin.

prothrombotic risk factor with carriers of combined prothrombotic defects and subjects without genetic risk factors. To determine their independent contributions to the risk of recurrent VTE single prothrombotic risk factors, 2 or more combined prothrombotic risk factors, gender and the presence of triggering factors (ie, recent immobilization, surgery, trauma, plaster casts, bacterial or viral infections, and/or the intake of oral contraceptives) were additionally analyzed by multivariate logistic procedure (adjusted odds ratio [OR] and 95% confidence interval [CI]). In addition, the relative risk (RR) and 95% CI were calculated to compare carriers of prothrombotic risk factors with patients without identified genetic risk and single defects with combined defects respectively. *P* values < .05 were considered significant.

Results

Clinical characteristics and prevalence of prothrombotic defects in the total study population

Clinically confirmed and imaging-confirmed thrombotic manifestations at first symptomatic onset were femoral vein thrombosis (n = 109), isolated calf vein thrombosis (n = 49), cerebral venous sinus thrombosis (n = 35), renal venous thrombosis (n = 31), iliac and inferior caval vein thrombosis (n = 37), portal vein thrombosis (n = 19), splenic vein thrombosis (n = 4), mesenteric vein thrombosis (n = 3), and isolated pulmonary embolism (n = 14).

With reference to all 301 symptomatic children with a first spontaneous VTE, one single hereditary risk factor was present in 58.5% (n = 176), whereas combined prothrombotic risk factors were found in 20.6% (n = 62). In 63 patients (20.9%), no established risk factor predisposing to venous vascular accidents

could be diagnosed so far. Inherited prothrombotic defects found in the study population are shown in Tables 1 and 2.

Subgroup of patients with recurrent thromboembolic episodes: characteristics and prevalence of prothrombotic conditions

The 301 pediatric patients with a first spontaneous VTE not associated with one of the predefined acquired risk factors were followed prospectively for a median of 7 years (range, 6 months to 15 years) after withdrawal of anticoagulation to determine the frequency of recurrent VTE. Twenty-four patients (8.0%) were observed for 2 years, 81 (26.9%) for 5 years, 90 (30.0%) for 7 years, 63 (20.9%) for 10 years, and 43 (14.3%) for more than 15 years.

Of the 301 patients, 64 (male, n = 24; female, n = 40) experienced a first recurrent VTE at a median of 3.5 years (range, 7 weeks to 15 years) after discontinuation of anticoagulation, representing an incidence of 21.3%. The time interval from withdrawal of anticoagulation to recurrence was up to 6 months in 12 children (18.8%), 7 to 12 months in 8 (12.5%), 13 to 23 months in 7 (10.9%), 2 to 4 years in 16 (25.0%), 5 to 10 years in 18 (28.1%), and 15 years in 3 (4.7%).

Comparison of the site of recurrent VTE with that of first manifestation revealed an ipsilateral identical thrombus location in 35 (54.7%) of the 64 subjects, diagnosed only if a new intraluminal filling defect was found within the lumen of a vein compared with the previous tests. Ipsilateral proximal vascular occlusion (first thrombosis: isolated calf vein thrombosis; recurrence: femoral and iliac vein thrombosis) occurred in a further 10 patients (15.6%),

Table 2. Prevalence of combined prothrombotic defects in childhood venous thrombosis in relation to recurrence status

Combined defects (n = 62)	Prevalence n (%) in all patients (n = 301)	Prevalence n (%) in patients without recurrent VTE (n = 237)	Prevalence n (%) in patients with recurrent VTE (n = 64)	Prevalence (%) of recurrent VTE in carriers
FV 1691AA	7 (2.3)	5 (2.1)	2 (3.1)	2/7 (28.6)
FV 1691AA/GA and Elevated Lp(a)	39 (12.9)	15 (6.3)	24 (37.5)	24/39 (61.5)
Protein S def	16	6	10	
Antithrombin def	10	4	6	
Protein C def	6	3	3	
PT 20210GA	3	—	3	
Elevated Lp(a) and Protein C def	4	2	2	
PT 20210GA and Protein C def	12 (4.0)	9 (3.4)	4 (6.3)	4/12 (33.3)
PT 20210GA and Antithrombin def	7	6	1	
PT 20210GA and Protein S def	3	1	2	
Protein S def	1	—	1	
Protein C def and Protein S def	4 (1.3)	4 (1.7)	—	—
Protein S def	4	4	—	
Total	62 (20.6)	32 (13.5)	30 (46.9)	30/62 (48.4)

VTE indicates venous thromboembolism; FV, factor V; Lp(a), lipoprotein (a); def, deficiency; PT, prothrombin.

and contralateral leg involvement in 5 patients (7.8%). Two children (3.1%) with initial renal venous thrombosis experienced calf and femoral VTE, and in a further 2 patients (3.1%) femoral vein thrombosis followed isolated calf vein thrombosis. Isolated pulmonary embolism was found in 8 subjects (12.5%) with femoral vein thrombosis at first clinical manifestation (> 12 months after first thrombotic onset and > 6 months after withdrawal of anticoagulation). In a further 2 patients (3.1%) femoral vein thrombosis followed portal vein occlusion.

Of the 64 patients with recurrent VTE, 31 (48.4%) had one prothrombotic risk factor for venous thrombosis, 30 (46.9%) had 2 or more prothrombotic risk factors, 2 were homozygous carriers of the FV gene mutation, and in 3 subjects (4.8%) no risk factor could be identified so far. As shown in Tables 1 and 2, the highest recurrence rate was observed among homozygous carriers of the FV G1691A mutation, and none of the 64 children with a history of recurrent VTE was diagnosed with protein S deficiency.

When children carrying one risk factor were compared with pediatric patients with no prothrombotic risk factor, the RR of recurrent VTE was found to be 4.0 (95% CI, 1.2-13.2) in heterozygous and 6.0 (95% CI, 1.2-30.0) in homozygous carriers of the FV mutation, 5.3 (95% CI, 1.4-20) in protein C-deficient children, 3.8 (95% CI, 0.7-20.3) in carriers of the PT G20210A mutation, 2.6 (95% CI, 0.7-9.9) in pediatric patients with elevated Lp(a), and 4.2 (95% CI, 0.5-33.3) in children with antithrombin deficiency. Although recurrent VTE occurred in 17.6% (31 of 176) of patients with one risk factor, 48.4% (30 of 62) of children carrying combined prothrombotic defects had experienced recurrent VTE. In children carrying combined defects the RR of experiencing VTE episodes was 10.6 (95% CI, 3.2-31.6) compared with pediatric patients with no prothrombotic risk factor, and 2.7 (95% CI, 1.8-4.1) compared with patients carrying one single prothrombotic risk factor. Prevalence rates of combined prothrombotic defects are shown in detail in Table 2.

As shown in Figure 2, the cumulative thrombosis-free survival with respect to recurrent VTE episodes is significantly reduced in pediatric patients with combined defects compared with subjects carrying one single risk factor only or no prothrombotic risk factor ($P < .0001$; chi-square, 42.2).

In 50.0% of patients ($n = 32$) recurrent VTE occurred spontaneously, whereas acquired risk factors known to be associated with an increased risk of VTE were found in the remaining 32 children (immobilization, $n = 8$; surgery, $n = 7$; trauma, $n = 9$; intake of

oral contraceptives [females], $n = 8$). Acquired risk factors clinically documented during the regular follow-up sessions were also present at least once in 113 of the remaining 237 patients not suffering a second thrombotic event so far (immobilization, $n = 37$; surgery, $n = 17$; trauma, $n = 29$; plaster casts, $n = 6$; infection, $n = 16$; intake of oral contraceptives [females], $n = 7$; pregnancy, $n = 1$).

To determine their independent contribution to the risk of recurrent VTE, gender and the presence of inherited thrombophilic defects or acquired prothrombotic risk factors were analyzed by multivariate logistic procedure. However, compared to pediatric patients with no prothrombotic risk factor, only the presence of at least one prothrombotic risk factor (OR, 4.6; 95% CI, 2.3-9.0; $P < .0001$), and 2 or more combined prothrombotic defects markedly increased the risk of recurrent VTE in the pediatric patients investigated in the present study (OR, 24.0; 95% CI, 5.3-108.7; $P < .0001$). Neither gender (male: OR 1.5; 95% CI, 0.8-2.9; $P = .2$) nor the presence of an acquired predisposing factor (OR, 0.86; 95% CI, 0.45-1.6; $P = .7$) influenced the risk of recurrent VTE in these patients.

There were no significant differences among the groups of patients carrying different prothrombotic defects with respect to gender, age at first episode of VTE, time interval between first and recurrent VTE, or site of thrombotic manifestations.

Discussion

The contribution of various hereditary hemostatic abnormalities to the risk of VTE has been well established. In addition, elevated Lp(a) concentrations have recently been identified as a further inherited prothrombotic risk factor in children and adults.^{17,21} As shown in previous reports and by the data presented here, carriers of these prothrombotic risk factors are at high risk of developing first thrombotic events during childhood and early adolescence.^{11,17,18}

Furthermore, it is well known that patients who have experienced symptomatic VTE remain at risk for recurrent VTE even in childhood and early adolescence²² and after withdrawal of adequate anticoagulant treatment. In a long-term follow-up study of a consecutive series of adult patients with a first episode of deep venous thrombosis, the cumulative incidence of recurrent VTE exceeded 30% over a period of 8 years.¹⁶ The risk of recurrent VTE was reported by Simioni et al¹² to be significantly higher in carriers of the FV G1691A gene mutation compared with normozygous subjects. However, these findings were not confirmed by other investigators.^{13,15} In addition, evidence is given that combined inheritance of prothrombotic risk factors (ie, genetic recombination of FV G1691A with deficiencies of protein C,¹¹ S, and antithrombin^{10,12} as well as combination with the PT G20210A variant,^{13,17} or enhanced Lp(a) concentrations¹⁷) further increase not only the manifestation of early vascular accidents but also the risk of recurrent VTE.

Data of the prospective study presented here clearly show that the risk of recurrent VTE in children with a first symptomatic episode of VTE not associated with a secondary cause of thrombosis is significantly higher in patients carrying prothrombotic risk factors. The highest risk was observed in children with 2 or more combined prothrombotic risk factors compared with children without these defects. The majority of patients with recurrent VTE relapsed within a median of 3.5 years after withdrawal of anticoagulation, with a high rate of 31% within the first 12 months and 87% after 7 years follow-up. Thus, the early recurrence rate found in this study was similar to that recorded in adults²³ but higher than the 30% incidence rate of recurrent VTE recorded over an 8-year period.¹⁶

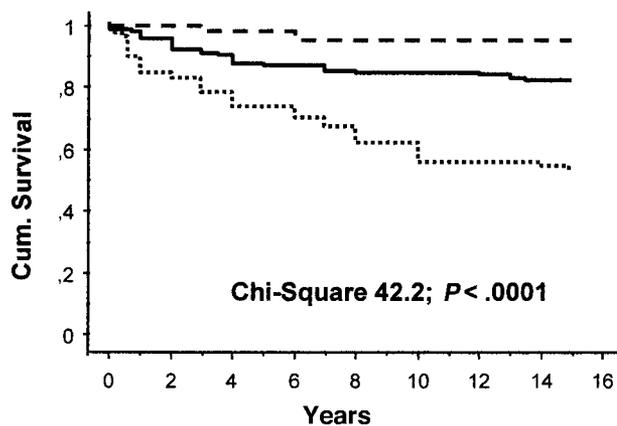


Figure 2. Cumulative recurrent-free survival in pediatric patients with combined prothrombotic risk factors compared with subjects carrying one single prothrombotic risk factor and no prothrombotic defect. Dotted line indicates combined defects (30/62); solid line, single defects (31/176); and dashed line, with out defects (3/63).

Multivariate logistic regression analysis moreover revealed that recurrent VTE in this group of pediatric patients was not additionally influenced by gender or by exogenous triggering factors (ie, immobilization, surgery, trauma, or oral contraceptives). The predefined exogenous triggering factors were present at least once in 113 of the remaining 237 pediatric patients not experiencing a second thrombotic event so far. This finding is similar to the distribution of risk factors in the patients with recurrent VTE. In addition, it was difficult in the cohort presented here to weight the different exogenous risk factors against each other with respect to their individual contribution to childhood recurrent VTE. Because we do not yet know why similar acquired risk factors promote recurrent VTE in some children and not in others, we suggest that there must be additional, still unknown risk factors. On the one hand, unless the role of different acquired triggering factors in childhood recurrent VTE is clarified, recommendations on secondary thromboprophylaxis based on these exogenous risks are not justified. On the other hand, data of this study are clinically important in indicating that a selected subgroup of pediatric patients with combined prothrombotic risk factors in the absence of further secondary causes of thrombosis carries a high risk of recurrent VTE, so that children of this selected subgroup might be candidates for early long-term anticoagulant therapy. In contrast to the data presented by Eichinger et al¹³ and by de Stefano and coworkers,¹⁵ but similar to those of Simioni et al,¹² recurrent VTE

in these preselected pediatric patients was more frequently observed in subjects carrying the FV G1691A mutation, either in its heterozygous or its homozygous form, or combined with further prothrombotic risk factors than in children with no identified prothrombotic trait.

In summary, with respect to recurrent VTE and the presence of prothrombotic risk factors, the results presented here can be applied to the majority of Caucasian pediatric patients with a first symptomatic onset of confirmed VTE occurring in the absence of further acquired secondary causes of vascular occlusion. Comprehensive screening for prothrombotic risks is, therefore, indicated in early symptomatic patients. In addition, because the prophylactic effect of long-term anticoagulant treatment in the presence of single or combined genetic defects remains a matter of controversial debate, particularly in childhood thrombosis, this issue should be assessed in a large-scale prospective study.

Acknowledgments

We thank all technicians from the participating laboratories, in particular Ruth Bäumer, Margit Käse, Christiane Schettler, and Doris Böckelmann for excellent technical assistance. In addition, we thank Dr A. Heinecke for his help in statistical analysis and Susan Griesbach for help in editing this manuscript.

References

- Kniffin WD, Baron JA, Barrett J, et al. The epidemiology of diagnosed pulmonary embolism and deep venous thrombosis in the elderly. *Arch Intern Med.* 1994;154:861-866.
- Lane DA, Mannucci PM, Bauer KA, et al. Inherited thrombophilia: Part 1. *Thromb Haemost.* 1996;76:651-662.
- Lane DA, Mannucci PM, Bauer KA, et al. Inherited thrombophilia: Part 2. *Thromb Haemost.* 1996;76:824-834.
- Bertina RM, Koelemann BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature.* 1994;396:64-67.
- Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood.* 1996;88:3698-3703.
- Schulman S, Rhedin AS, Lindmarker P, et al. A comparison of six weeks with six months of oral anticoagulant therapy after a first episode of venous thromboembolism. *N Engl J Med.* 1995;332:1661-1665.
- Salomon O, Steinberg DM, Zivelin A, et al. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism—prevalence and risk assessment. *Arteriol Thromb Vasc Biol.* 1999;19:511-518.
- Boven van HA, Reitsma PH, Rosendaal FR, et al. Factor V Leiden (FVR506Q) in families with inherited antithrombin deficiency. *Thromb Haemost.* 1996;75:417-421.
- Koelemann BPC, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. *Blood.* 1994;84:1031-1035.
- Zöllner B, Berntsdotter A, Frutos de PG, Dahlbäck B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. *Blood.* 1995;85:3518-3523.
- Ehrenforth S, von Depka Prondzinski M, Aygören-Pürsün E, Nowak-Göttl U, Scharrer I, Ganzer A. Study of the prothrombin gene 20210 GA variant in FV:Q506 carriers in relationship to the presence or absence of juvenile venous thromboembolism. *Arterioscler Thromb Vasc Biol.* 1999;19:276-280.
- Simioni P, Prandoni P, Lensing AWA, et al. The risk of recurrent venous thromboembolism in patients with an Arg506Gln mutation in the gene for factor V (Factor V Leiden). *N Engl J Med.* 1997;336:399-403.
- Eichinger S, Pabinger I, Stümpflen A, et al. The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. *Thromb Haemost.* 1997;77:624-628.
- Lindmarker P, Schulman S, Sten-Linder M, et al. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. *Thromb Haemost.* 1999;81:684-689.
- Stefano de V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. *N Engl J Med.* 1999;341:801-806.
- Prandoni P, Lensing AWA, Cogo A, et al. The long-term clinical course of acute deep venous thrombosis. *Ann Intern Med.* 1996;125:1-7.
- Nowak-Göttl U, Junker R, Hartmeier M, et al. Increased lipoprotein (a) is an important risk factor for venous thrombosis in childhood. *Circulation.* 1999;100:743-748.
- Nowak-Göttl U, Sträter R, Heinecke A, et al. Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin and methylenetetrahydrofolate reductase are risk factors of ischaemic stroke in childhood. *Blood.* 1999;94:3678-3682.
- Junker R, Koch HG, Auberger K, Münchow N, Ehrenforth S, Nowak-Göttl U. Prothrombin G20210A gene mutation and further prothrombotic risk factors in childhood thrombophilia. *Arterioscler Thromb Vasc Biol.* 1999;19:2568-2572.
- Nowak-Göttl U, Wermes C, Junker R, et al. Prospective evaluation of the thrombotic risk in children with acute lymphoblastic leukemia carrying the MTHFR TT677 genotype, the prothrombin G20210A variant, and further prothrombotic risk factors. *Blood.* 1999;93:1595-1599.
- Von Depka Prondzinski M, Nowak-Göttl U, Eisert R, et al. Increased lipoprotein (a) levels as an independent risk factor for venous thromboembolism. *Blood.* 2000;96:3364-3368.
- Streif W, Andrew M, Marzinotto V, et al. Analysis of Warfarin therapy in paediatric patients: a prospective cohort study of 319 patients. *Blood.* 1999;94:3007-3014.
- Kearon C, Gent M, Hirsh J, et al. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. *N Engl J Med.* 1999;340:901-907.

Appendix

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