Prothrombin 20210A Mutation

A Mild Risk Factor for Venous Thromboembolism but Not for Arterial Thrombotic Disease and Pregnancy-Related Complications in a Family Study

Ivan Bank, MD, PhD; Eduard J. Libourel, MD; Saskia Middeldorp, MD, PhD; Elisabeth C. M. van Pampus, MD, PhD; Maria M. W. Koopman, MD, PhD; Karly Hamulyák, MD, PhD; Martin H. Prins, MD, PhD; Jan van der Meer, MD, PhD; Harry R. Büller, MD, PhD

Background: The prothrombin 20210A mutation has been associated with an increased risk of venous thromboembolism (VTE). Its relationship with arterial disease and pregnancy-related complications is, however, still uncertain. The aim of this study was to estimate the incidences of first venous and arterial thrombotic events and pregnancy-related complications in relatives of patients with the mutation.

Methods: After clinical classification, the presence of the mutation was determined in first-degree relatives of consecutive patients with the mutation and a history of VTE or premature atherosclerosis. Relatives with and without the mutation were compared.

Results: Of all relatives, 204 (50%) were heterozygous, 5 were homozygous, and 198 had a normal genotype. The annual incidence of a first episode of VTE was 0.35% and 0.18% in carriers and noncarriers, respectively (odds ratio [OR], 1.9; 95% confidence interval [CI], 0.9-4.1); the annual incidence of a first arterial thrombosis was 0.22% and 0.15% in carriers and noncarriers, respectively (OR, 2.3; 95% CI, 0.8-6.3). The annual incidence of a first myocardial infarction was 0.14% (95% CI, 0.05%-0.23%) and 0.05% (0.01%-0.14%) in carriers and noncarriers, respectively (OR, 4.7; 95% CI, 1.0-22.5; P = .06). In particular, homozygous carriers were at increased risk of VTE (OR, 6.0; 95% CI, 1.3-27.2), whereas a history of VTE in the proband influenced the risk of VTE in the relatives. Women with the mutation did not experience significantly more pregnancy-related complications than their relatives with a normal genotype.

Conclusions: The prothrombin mutation is a mild risk factor for VTE within families of carriers but does not seem to play an important role in arterial thrombotic disease, with the exception of myocardial infarction, or in pregnancy-related complications.

Arch Intern Med. 2004;164:1932-1937

Since its identification as a risk factor for venous thromboembolism (VTE) in 1996, there is growing interest in the prothrombin 20210A mutation and its role in cardiovascular and pregnancy-related diseases.1 However, there are some unresolved issues.

Early reports described the relationship between the prothrombin 20210A mutation and (recurrent) venous and arterial thrombosis.2-7 More recent case-control studies suggested that this mutation is only a weak risk factor, inducing mainly VTE in combination with other (transient or inherited) risk factors.8-13 Its relationship with arterial thrombosis was also questioned.14-17

Thrombophilic factors might influence perfusion of the placenta, inducing spontaneous abortion or intrauterine growth retardation, or predispose to preeclampsia.18 Whether the prothrombin mutation has such an effect remains, however, a matter of debate.19-21 A recently performed meta-analysis of case-control studies indicated that women carrying the prothrombin 20210A mutation have an approximately 2-fold increase in risk of recurrent fetal loss, compared with noncarriers.22

Because of these conflicting data and the lack of knowledge about absolute risks within families of patients with the prothrombin 20210A mutation, we performed a large retrospective family cohort study. To assess the thrombotic and pregnancy-related risks in families, we studied first-degree relatives of consecutive patients with the prothrombin 20210A mutation and proven VTE or premature atherosclerosis.

Methods

STUDY DESIGN

From May 1, 1998, to March 15, 2003, first-degree relatives of consecutive patients (pro-
bands) with documented VTE or premature atherosclerosis (any arterial thrombotic event before the age of 50 years) and the prothrombin 20210A mutation were enrolled in the study. Screening for thrombophilia was performed in all patients with VTE or premature atherosclerosis, independent of age, prognosis, or suitability for participation in this study.

We obtained a detailed medical history with special emphasis on previous episodes of venous and arterial thrombosis and pregnancy-related complications; transient risk factors for VTE, including surgery, immobilization, pregnancy, and use of oral contraceptives; and known risk factors for arterial disease, such as smoking, diabetes mellitus, hypertension, and hyperlipidemia.

The carrier status of relatives was determined after the medical history had been documented, and individuals were classified as being symptomatic or asymptomatic for venous or arterial thrombosis if they had experienced events as defined in the following section; the same was done for pregnancy-related complications in women. Relatives who did not carry the prothrombin 20210A mutation were used as controls. Probands were not included in the analysis.

The study was approved by the local ethics committees, and participants provided written informed consent.

DEFINITIONS

Venous thromboembolism was considered to have occurred if it had been confirmed by venography, ultrasonography, or impedance plethysmography in cases of deep venous thrombosis, and ventilation-perfusion lung scanning, angiography, or computed tomography in cases of pulmonary embolism. If an episode of VTE had not been diagnosed by objective methods at a time when these were not routinely used, it was classified as an event only if it had been treated with vitamin K antagonists or low-molecular-weight heparin for at least 6 weeks.

Coronary and peripheral arterial disease had to be symptomatic and angiographically proved, while myocardial infarction had to be diagnosed according to clinical, enzymatic, and electrocardiographic criteria. Ischemic stroke was defined as the onset of rapidly developing symptoms and signs of loss of cerebral function that lasted at least 24 hours and had no apparent nonvascular cause. Furthermore, it had to be confirmed by means of computed tomography or magnetic resonance imaging. If a cerebrovascular event resolved completely within 24 hours without signs of cerebral lesions on scanning results, it was classified as a transient ischemic attack.

Episodes of increased risk of VTE included recent surgery, trauma, immobilization for more than 7 days, pregnancy, postpartum period, malignancy, and use of oral contraception or hormone therapy. Venous thromboembolism was considered to be related to such an episode when it had occurred within 3 months. A relationship with the use of oral contraception or hormone therapy was defined as an event occurring during exposure. Known risk factors for arterial thrombosis were also recorded and included past and current smoking habits and the presence of diabetes mellitus, hyperlipidemia, and/or hypertension.

An abortion was defined as fetal loss after the eighth week of gestation but before the 20th week of gestation. The HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome and pregnancy-related hypertension were defined as used in clinical practice.

When appropriate, patient’s charts were reviewed, or treating physicians or general practitioners were contacted. All events were classified before knowledge of the genotype.

MUTATION ANALYSIS

The DNA analysis was performed by polymerase chain reaction amplification of genomic DNA extracted from peripheral leukocytes by standard methods, followed by digestion with the restriction enzyme HindIII to identify the variant allele at position 20210 of the prothrombin gene.

STATISTICAL ANALYSIS

Annual incidences of first episodes of VTE and arterial thrombotic events in the carrier and noncarrier groups were calculated by dividing the number of events by observed years. These incidences were also assessed for the age groups 15 to 30, 31 to 45, 46 to 60, and greater than 60 years. Observation years were defined as years since the age of 15 years until the date of inclusion or until the date of the first thrombotic event. However, if a relative had experienced both arterial and venous events, separate observation years were used for analysis: all years until the first venous event were taken for VTE, whereas all years until the first arterial thrombotic event were calculated for arterial thrombotic disease.

Odds ratios (ORs) were used to estimate the relative risk. Multivariate analysis was used for the determination of adjusted arterial thrombotic risk, and conditional logistic regression was applied to estimate the OR. We adjusted for known risk factors for arterial disease, ie, smoking, diabetes mellitus, hypertension, and hyperlipidemia. Conditional logistic regression was also used to analyze the role of a family history in the occurrence of VTE and arterial thrombosis.

Relatives with the mutation were compared with relatives with a normal genotype. The incidence of spontaneous abortion was calculated by dividing the number of abortions by the total number of pregnancies in carriers and noncarriers, and by the number of women who had been pregnant. Incidences of pregnancy-related complications (pregnancy-induced hypertension and HELLP syndrome) were calculated by dividing the total number of events by the total number of pregnancies of greater than 20 weeks of gestation. The 95% confidence intervals (CIs) were calculated according to normal approximation of the binomial distribution. Statistical significance was determined by χ² test and paired, 2-tailed t test.

RESULTS

STUDY POPULATION

The pedigrees of 123 probands, all heterozygous carriers of the prothrombin 20210A mutation (94 patients with objectively documented VTE, 27 patients with premature atherosclerosis, and 2 patients with both VTE and premature atherosclerosis) disclosed 665 first-degree relatives older than 15 years. Of these relatives, 124 had died before this study began. For 25 of them (20.2%), the possible cause of death was reported to be of cardiovascular origin. Of all relatives who died, 7 (5.6%) had possibly experienced VTE and 18 (14.5%) had possibly experienced an arterial thrombotic event during their lifetime. Another 134 relatives did not participate because of various reasons, including reluctance to undergo assessment of genetic disorders, inability to give informed consent, and residence outside the Netherlands. A total of 407 first-degree relatives were included at the time of analysis (response rate of living first-degree relatives, 75%; overall response rate, 61%).

Of the participants, 209 (51%) were found to be carriers of prothrombin 20210A mutation (204 heterozygous, 5 homozygous) and 198 were noncarriers (Table 1).
INCIDENCE OF VTE

Thirty-two relatives had experienced at least 1 episode of VTE. Of these, 21 were carriers and 11 were noncarriers (Table 2). The absolute annual incidence of a first episode of VTE in all carriers was 0.35% (95% CI, 0.21%-0.53%), compared with 0.18% (0.09%-0.33%) in noncarriers (OR, 1.9 [95% CI, 0.9-4.1]) (Table 2). Homozygous carriers had an annual incidence of VTE of 1.10 (95% CI, 0.13-3.97) (OR compared with noncarriers, 6.0 [95% CI, 1.3-27.2]).

Carriers had their first event of VTE at a younger age than noncarriers (mean±SD, 35±17 years vs 43±14 years; P=.001). Twelve carriers developed VTE before the age of 30 years, compared with 2 of the noncarriers (annual incidence, 0.44% [95% CI, 0.23%-0.76%] and 0.08% [95% CI, 0.01%-0.28%], respectively; OR, 5.7 [95% CI, 1.3-25.5]) (Table 2).

Among the relatives of probands who were included with VTE, the annual incidence of a first episode of VTE in carriers was 0.41% (95% CI, 0.24%-0.65%), compared with 0.17% (95% CI, 0.07%-0.33%) in noncarriers (OR, 2.4 [95% CI, 1.1-5.6]). After correction for family history, the OR was 2.4 (95% CI, 1.0-5.8) (P=.04).

In carriers, 38% of VTE occurred spontaneously, whereas all events in noncarriers were related to transient risk factors (Table 3). The incidences of VTE associated with surgical proceedings, trauma, or immobilization did not differ between carriers and noncarriers (OR, 0.8 [95% CI, 0.3-2.5]).

The absolute incidence of VTE among women who used oral contraceptives and carried the prothrombin 20210A mutation was 0.2% (95% CI, 0.0%-0.9%), whereas the incidence of pregnancy-related VTE was 2.8% (95% CI, 1.0%-6.0%). These incidences did not differ significantly from those observed in noncarriers. No women developed VTE while using hormone therapy.

INCIDENCES OF FIRST ARTERIAL THROMBOTIC EVENTS

Table 4 shows the frequencies and incidences of first arterial thrombotic events in carriers and noncarriers. Fourteen first events occurred in carriers, compared with 9 events in noncarriers. About 43% of all relatives (carriers and noncarriers) who developed arterial events experienced their first arterial thrombotic event before the age of 50 years. Adjusted for major risk factors for cardiovascular disease, the observed difference between the 2 groups did not reach statistical significance for a first arterial thrombotic event (OR, 2.3 [95% CI, 0.8-6.3]); additional correction for family history did not change this result (OR, 1.9 [95% CI, 0.6-5.9]). However, the annual incidence of a first myocardial infarction was 0.14% (95% CI, 0.05%-0.23%) and 0.05% (95% CI, 0.01%-0.14%) in carriers and noncarriers, respectively (OR, 4.7 [95% CI, 1.0-22.5]; P=.06). Carriers younger than 60 years were at increased risk for a first arterial thrombotic event compared with noncarriers (OR, 1.0 [95% CI, 0.3-3.3]); for carriers older than 60 years, the observed odds ratio did not reach statistical significance (OR, 2.0 [95% CI, 0.6-6.5]). The age-specific incidences of a first arterial thrombotic event are shown in the Figure.

PREGNANCY-RELATED COMPLICATIONS

Of all participating women, 156 had ever been pregnant. Of them, 81 were carriers. Table 5 shows incidences of pregnancy-related complications. Thirteen percent of pregnancies in carriers and 12.3% of pregnancies in noncarriers ended in spontaneous abortion. The risk for women carrying the prothrombin 20210A mutation appeared to be slightly higher, but the observed difference did not reach statistical significance (OR, 1.3 [95% CI, 0.7-2.6]). Incidences of recurrent spontaneous abortions in carriers and noncarriers were not different (OR, 0.9 [95% CI, 0.3-3.3]).
In this large retrospective family study, we found that among relatives of patients with the mutation, carriers of the prothrombin 20210A allele had an annual incidence of 0.35% (95% CI, 0.21%-0.53%) for a first episode of VTE and 0.22% (95% CI, 0.11%-0.34%) for a first arterial thrombotic event. Compared with the observed incidences of first events in noncarriers, there was a trend to higher incidences in carriers, but the observed differences did not reach statistical significance. However, within families of probands who had experienced VTE, carriers of the mutation were at increased risk for VTE compared with noncarriers (OR, 2.4 [95% CI, 1.0-5.8]; P = .04), indicating that for the risk of VTE in carriers, the family history is of importance, as was previously shown for factor V Leiden carriers. Furthermore, in particular, homozygous carriers of the mutation had an increased risk of VTE, although this finding has to be interpreted with caution because of the small number of subjects we were able to study.

In contrast to other reports, we were unable to demonstrate an increased risk of VTE induced by transient risk factors, especially not related to pregnancy or use of oral contraceptives.5,27,28

The observed high annual incidence of VTE in carriers (0.44% [95% CI, 0.23-0.76]) younger than 30 years is striking. It appears higher than in individuals younger than 30 years carrying the factor V Leiden mutation (0.25% [95% CI, 0.12%-0.49%]) but somewhat lower than

---

**Table 3. Relationship of First Episodes of VTE to Established Risk Factors**

<table>
<thead>
<tr>
<th></th>
<th>No Prothrombin Mutation (n = 11)</th>
<th>Prothrombin Mutation (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous, No. (%)</td>
<td>0</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Pregnancy related</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of episodes</td>
<td>170</td>
<td>215</td>
</tr>
<tr>
<td>No. with VTE</td>
<td>2*</td>
<td>6†</td>
</tr>
<tr>
<td>Incidence, % (95% CI)</td>
<td>1.2 (0.1-4.2)</td>
<td>2.8 (1.0-6.0)</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>2.4 (0.5-121)</td>
<td></td>
</tr>
</tbody>
</table>

**Operation, trauma, immobilization**

<table>
<thead>
<tr>
<th></th>
<th>Noncarriers</th>
<th>Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of episodes</td>
<td>292</td>
<td>305</td>
</tr>
<tr>
<td>No. with VTE</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Incidence, % (95% CI)</td>
<td>2.4 (1.0-4.9)</td>
<td>2.0 (0.8-4.2)</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>0.8 (0.3-2.5)</td>
<td></td>
</tr>
</tbody>
</table>

**Oral contraceptives**

<table>
<thead>
<tr>
<th></th>
<th>Noncarriers</th>
<th>Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years of use</td>
<td>655</td>
<td>660</td>
</tr>
<tr>
<td>No. with VTE</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Incidence/y of use, % (95% CI)</td>
<td>0.3 (0.0-1.1)</td>
<td>0.2 (0.0-0.9)</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>0.5 (0.1-5.5)</td>
<td></td>
</tr>
</tbody>
</table>

---

Age-specific annual incidence of a first occurrence of an arterial thrombotic event. The limit lines represent 95% confidence intervals.

---

**Table 4. Frequencies and Incidences of First Episodes of Arterial Thrombotic Event in First-Degree Relatives of Probands With Prothrombin 20210A Mutation**

<table>
<thead>
<tr>
<th></th>
<th>No. of Individuals With Event</th>
<th>No. of Observation Years</th>
<th>Incidence/y, % (95% CI)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>No prothrombin mutation</td>
<td>3</td>
<td>6174</td>
<td>0.05 (0.01-0.14)</td>
<td>2.9 (0.8-11.0)</td>
</tr>
<tr>
<td></td>
<td>Prothrombin mutation</td>
<td>9</td>
<td>6412</td>
<td>0.14 (0.05-0.23)</td>
<td>2.9 (0.8-11.0)</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>No prothrombin mutation</td>
<td>1</td>
<td>6202</td>
<td>0.02 (0-0.48)</td>
<td>1.0 (0.2-4.8)</td>
</tr>
<tr>
<td></td>
<td>Prothrombin mutation</td>
<td>1</td>
<td>6481</td>
<td>0.02 (0-0.46)</td>
<td>1.0 (0.1-16.7)</td>
</tr>
<tr>
<td>TIA</td>
<td>No prothrombin mutation</td>
<td>3</td>
<td>6190</td>
<td>0.04 (0-0.10)</td>
<td>1.0 (0.1-15.3)</td>
</tr>
<tr>
<td></td>
<td>Prothrombin mutation</td>
<td>3</td>
<td>6457</td>
<td>0.05 (0-0.10)</td>
<td>1.3 (0.3-7.1)</td>
</tr>
<tr>
<td>Peripheral arterial thrombotic event</td>
<td>No prothrombin mutation</td>
<td>2</td>
<td>6150</td>
<td>0.03 (0-0.08)</td>
<td>0.5 (0-5.2)</td>
</tr>
<tr>
<td></td>
<td>Prothrombin mutation</td>
<td>1</td>
<td>6452</td>
<td>0.02 (0-0.46)</td>
<td>0.4 (0-5.8)</td>
</tr>
<tr>
<td>Any first arterial thrombotic event</td>
<td>No prothrombin mutation</td>
<td>9</td>
<td>6110</td>
<td>0.15 (0.05-0.24)</td>
<td>1.5 (0.6-3.6)</td>
</tr>
<tr>
<td></td>
<td>Prothrombin mutation</td>
<td>14</td>
<td>6352</td>
<td>0.22 (0.11-0.34)</td>
<td>2.3 (0.8-6.3)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio; TIA, transient ischemic attack.

*Adjusted for smoking, diabetes mellitus, hypertension, and hyperlipidemia.
in young individuals with inherited antithrombin, protein C, or protein S deficiency (0.94% [95% CI, 0.58%-1.46%]). Although the possibility of selection as a cause of this observation cannot be completely ruled out, this is unlikely because all patients with VTE or premature atherosclerosis were screened for thrombophilia, independent of age or prognosis in all 3 participating centers, and all patients who were able to give informed consent were asked to participate.

Concerning arterial thrombotic risks, the annual incidences of first arterial events in carriers were comparable with those in noncarriers. However, carriers of the mutation had an almost 5-fold, borderline significant increase in the risk of a first episode of myocardial infarction compared with noncarriers. Our findings that arterial thrombotic risks were low and that almost half of all events occurred before the age of 50 years are similar to other observations.\(^{3,14,16,31,32}\)

Although our family study does not indicate that the prothrombin mutation is a risk factor for pregnancy-related complications in female relatives, it is notable that about one third of all carriers who had ever been pregnant had experienced a spontaneous abortion (OR compared with noncarriers, 1.3; 95% CI, 0.7-2.6). The incidences of abortions in both groups remained within the range known for the normal population.\(^3\)

Some methodologic aspects of our study warrant comment. First, to limit the potential for bias and misclassification, we used a standardized questionnaire, applied strict a priori defined criteria concerning classification of events, and obtained all information without knowledge of the genetic status. Second, we did not reach an optimal response rate. Nevertheless, we were able to obtain known causes of death of virtually all relatives not participating in the study, and there was no evidence that the observed incidences were underestimated. Third, the reported cardiovascular mortality rate was lower than in the general population, and it is known that life expectancy of individuals with other, even more penetrant inherited thrombophilic factors is normal.\(^{34,35}\) Although the reported prevalence of possible VTE in deceased relatives was 5.6%, it is unlikely that this has resulted in underestimation of the incidence of VTE in carriers. In a worst-case scenario in which all 7 deceased individuals with reported VTE are included as carriers, 50% of all deceased relatives would have been carriers, and life expectancy in these carriers would be decreased to 65 years, the annual incidence of a first episode of VTE would not differ (0.31% [95% CI, 0.19%-0.42%]). For any first arterial event in the same worst-case scenario, the annual incidence would increase only from 0.22% (95% CI, 0.11%-0.34%) to 0.34% (95% CI, 0.22%-0.44%). Thus, the inability to include deceased relatives does not materially affect our observations.

Finally, the clinical characteristics and risks of VTE in noncarriers did not differ from those of the general population or published family studies, indicating that noncarriers were appropriate for comparison.\(^30,37\)

We conclude that the prothrombin 20210A mutation is a mild risk factor for VTE in relatives of patients with this mutation and a history of VTE, but not in all carriers. In particular, homozygous carriers are at increased risk of VTE, and approximately 40% of all events of VTE occur spontaneously, whereas transient risk factors for VTE do not significantly increase the risk of VTE in carriers compared with noncarriers. Carriers have an almost 5-fold borderline increased risk of a first myocardial infarction compared with noncarriers, although it is unlikely that the prothrombin mutation plays a major role in the cause of other arterial thrombotic events or the occurrence of pregnancy-related complications within families of carriers with VTE or premature atherosclerosis.

Accepted for publication November 28, 2003.

This study was supported by grant 99.187 from the Dutch Heart Foundation, The Hague, the Netherlands.

We thank research nurses Mia Muller, RN, Jeannine van Suijlekom, RN, and Marja Voskuilen, RN, as well as Joost C. M. Meijers, PhD, head of the Laboratory of Vascular Medicine, for their excellent help.

Correspondence: Ivan Bank, MD, PhD, Department of Vascular Medicine, Academic Medical Center, F4-277, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands (I.Bank@amc.uva.nl).
REFERENCES