# Prothrombin gene 20210A mutation in Slovak population

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**Summary**

**Introduction**: Factor V Leiden (FVL) and prothrombin G20210A mutation (PTM) are the two most common genetic polymorphisms known to predispose to a first episode of venous thromboembolism (VTE). PTM is present in 2 % Caucasian population. The main aim of this study was to identify the PTM in the patients with positive history of thrombotic events vs. control subjects. **Materials and Methods**: The assessment of PTM was performed by the PCR analysis of the chromosomal DNA, which was isolated from the peripheral blood leukocytes. **Results**: Of the 2 274 patients included, 157 (6.9 %) were carriers of the PTM. The mutation was present only in 2.6 % (n = 8) of the 303 controls. The following clinical manifestations of PTM were analysed. We observed 123 venous thrombotic events, 46 arterial thrombosis and 14 spontaneous abortions. In this article we analyse other possible risk factors for thromboembolic events in patients with carriage of PTM. **Conclusions**: To our knowledge, this is the largest epidemiological study of PTM in Central Europe. Employing statistical analysis, we found relatively high prevalence of the PTM in both, the patients with positive thrombosis history (6.9 %), as well as in the control group (2.6 %). The risk of thrombosis by carriage of PTM is independent of age and gender. Study has shown relatively frequent presence of double carriership of PTM and factor V Leiden mutation (FVL).

**Key words**: mutation – population – prothrombin – thrombophilia – thrombosis

## Mutácia protrombínového génu 20210A v slovenskej populácii

**Súhrn**

**Úvod**: Mutácia faktora V Leiden (FVL) spolu a mutácia G20210A v protrombínovom géne (PTM) patria medzi 2 najčastejšie genetické polymorfizmy, ktoré predispozíciou pre rozvoj prevej epizódy venózneho tromboemblizmu (VTE). PTM sa vyskytuje v 2 % belošskej populácie. Hlavným cieľom tejto práce bolo zistiť prevalenciu PTM v populácii pacientov s anamnézou trombotickej príhody vs. zdravých kontrolách. **Materiál a metódy**: Za účelom posúdenie prítomnosti PTM bola realizovaná PCR analýza z DNA extrahovanej z periférnych leukocytov. **Výsledky**: Do štúdie bolo zaradených 2 274 pacientov, z nich 157 (6,9 %) malo prítomnú PTM. PTM mutácia bola prítomná u 2,6 % kontrol z celkového počtu 303 dobrovoľníkov. Analyzovali sme klinickú manifestáciu PTM. Pozorovali sme 123 venóznych trombóz, 46 artériových trombóz a 14 opakovaných spontánnych potratov. V tomto článku sme ďalej analyzovali ďalšie možné rizikové faktory rozvoja trombózy u pacientov s prítomnou PTM. **Záver**: Podľa našich vedomostí je toto najväčšia epidemiologická štúdia zameraná na výskyt PTM v strednej Európe. Za použitia štatistickej analýzy sme zistili relatívne vysoký výskyt PTM v populácii pacientov s anamnézou trombózy (6,9 %), ale aj u zdravých kontrol (2,6 %). Riziko trombózy je nezávislé od veku a pohlavia. Štúdia zároveň ukázala pomerne častý výskyt dvojitej prítomnosti PTM a FVL.

**Kľúčové slová**: mutácia – populácia – protrombín – trombóza

## Introduction

The prothrombin G20210A mutation (PTM) is the second most common inherited risk factor for thrombosis [1]. The prothrombin gene, located on chromosome 11 (11p11-q12), is a 21-kb gene containing 14 exons [2]. The PTM is a single nucleotide substitution of adenine for guanine in the 3´-untranslated region of the gene, first described by Poort et al in 1996 [3]. Many studies have shown elevated prothrombin levels in patients with PTM [3–9]. In addition to this finding, prothrombin level itself is a risk factor for thrombosis [3]. The initial Poort et al study [3] found that the mutation was present in 18 % of patients with a personal and family history of VTE and 6.2 % of unselected patients with first

time VTE, compared to 2.3 % of healthy controls. Subsequent studies show prevalence in healthy European and American individuals ranging from 1.2 to 4.6 % [10–15]. The mutation is less prevalent in non-Jewish, non-European populations [10,16–19].

The risk of VTE in women during pregnancy or oral contraceptive therapy appears to be increased in the presence of the PTM [20]. Martinelli et al [21] reported that the relative risk of deep vein thrombosis was increased 16.3-fold (95% CI, 3.4–79.1) in women with PTM who used oral contraceptives (OCPs) compared with non-carriers and non-users. And the relative risk of pregnancy and puerperal VTE in women with the PTM was 15.2 (95% CI, 4.2–52.6) [22].

Current research at our department is focused on platelet hyperaggregability and high activity of coagulation factor XI. We confirmed that these factors are associated with thrombosis and miscarriage [23–26].

The aim of the study was to detect the incidence of PTM in the patients with positive history of thrombotic events compared to the control group. In a more detailed value analysis, we evaluate the impact of this mutation on the clinical expression.

## Material and Methods

### Study population

The Ethical Committee of the Jessenius Faculty of Medicine, Comenius University approved the study. All study participants agreed to participate in the project and signed a written informed consent in accordance with the Declaration of Helsinki.

Data collection took place between September 2007 and September 2012. A detailed history regarding previous thrombotic episodes, a family history of thrombosis, and acquired risk factors such as cirrhosis, pregnancy, smoking, OCPs use, diabetes, hypertension, surgery, immobilization, injury, and malignant neoplasm was obtained from all patients and control subjects.

This study is comprised of patients (n = 2 274) with venous (n = 1 720) or arterial (n = 128) thrombosis and spontaneous abortion (n = 456). The miscarriage occurred in 220 patients during the first trimester of pregnancy (n = 74 recurrent pregnancy loss) and 102 patients during the second trimester (n = 31 recurrent pregnancy loss). 21 patients had recurrent pregnancy loss during the first and second trimester. The diagnosis of venous or arterial thrombosis was confirmed on ultrasonography, CT or angiography. Patients were initially examined and tested at the Department of Haematology and Transfusiology in Martin University Hospital. They were referred to undergo the thrombophilia screening as a part of differential diagnosis due to the thromboembolic event or spontaneous abortion. Most of the investigated parameters can be affected by acute thrombosis and anticoagulant therapy. Therefore, we used laboratory tests after discontinuation of the anticoagulant treatment. Patients were screened for Factor V Leiden (FVL), PTM, Protein C, Protein S, antithrombin III, factor VIII, factor IX, homocysteine, antiphospholipid syndrome and sticky platelet syndrome.

Furthermore, 303 randomly chosen healthy individuals were involved as control subjects. All control individuals were Caucasians of European origin, with negative personal and family history of the thrombosis without any chronic condition and any regular medication. The characteristics of the study population is presented in tab 1.

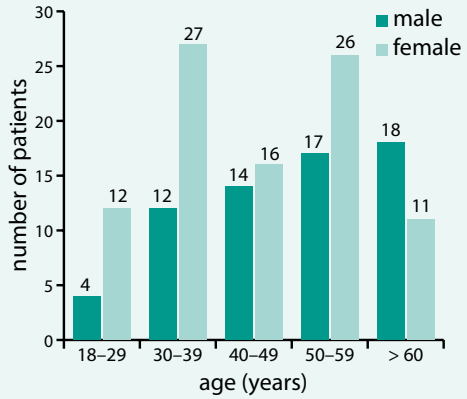
### Analysis of prothrombin gene

The blood drawn from antecubital vein, collected into vials prefilled with 400 µL 0.5 molar EDTA was used for the DNA analysis. Genomic DNA was extracted from peripheral blood leukocytes by SiMaxTM Genomic DNA Extraction kit (SBS Genetech Co., Ltd., China) according to the manufacturer's instructions. The strategy was direct detection of the 20210A allele in the prothrombin gene. A 345-bp fragment from exon 14 and the 3′-untranslated region of the prothrombin gene was amplified by polymerase chain reaction (PCR) using the primers 5′-TCTAGAAACAGTTGCCTGGC-3′ and a mutagenic primer 5´-ATAGCACTG GGAGCATTGAAG C-3´. Amplification was carried out in the following manner: initial denaturation at 95 °C for 3 minutes followed by 30 cycles of 95 °C for 30 seconds, 63 °C for 60 seconds, and 72 °C for 60 seconds. One microliter of HIND III restriction enzyme was added to the amplified DNA and incubated at 37 °C for 4 hours. The digested products (10 µL) were electrophoresed on 6% polyacrylamide gel until the blue dye front migrated at least 6 cm; 0.1% silver nitrate was used

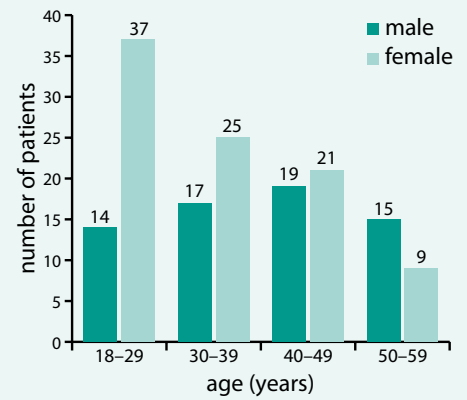
**Tab. 1. Characteristics of the study population**

| | patients | controls |
|---|---|---|
| **general characteristics** | | |
| total | 2 274 (100 %) | 303 (100 %) |
| men | 866 (38 %) | 115 (38 %) |
| average age (range), years | 37 (18–64) | 34 (18–59) |
| **type of event** | **n (%)** | |
| deep vein thrombosis | 1 233 (54.2 %) | |
| PTM | 60 (2.6 %) | |
| PTM + FVL | 22 (1 %) | |
| pulmonary embolism | 487 (21.4 %) | |
| PTM | 29 (1.3 %) | |
| PTM + FVL | 12 (0.5 %) | |
| arterial thrombosis | 128 (5.6 %) | |
| PTM | 37 (1.6 %) | |
| PTM + FVL | 9 (0.4 %) | |
| spontaneous abortions | 456 (20.1 %) | |
| PTM | 11 (0.4 %) | |
| PTM + FVL | 3 (0.2 %) | |

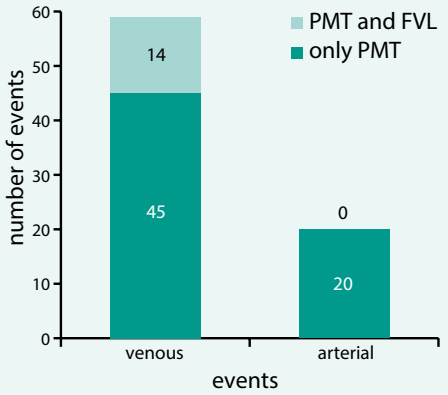
**Graph 1. Age distribution of patients with evidence of PTM and history of thromboembolism or abortions**



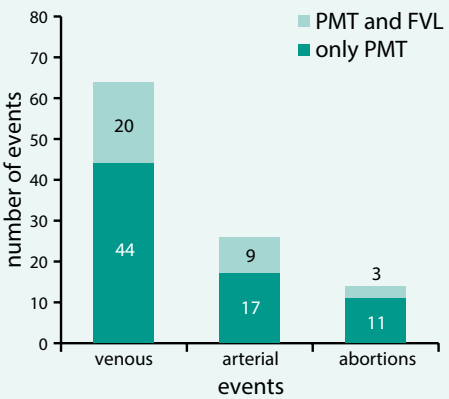

**Graph 2. Age distribution of patients with evidence of PTM according to the first thromboembolic event or abortion**




**Graph 3. Clinical manifestations according to gender – male**




**Graph 4. Clinical manifestations according to gender – female**




for staining. A heterozygous control was run with each batch of tests. An uncut amplified DNA and a wild-type DNA were also run with each batch of tests to ascertain the effectiveness of HIND III digestion.

## Statistics

We used Microsoft Office Excel for Mac 2011 (version 14.3.0) to do data analysis. Differences in the incidence of parameters were evaluated by Chi-Square test. The Fisher exact test was used in case of low representation (less than 5). P values less than 0.05 were considered statistically significant.

## Results

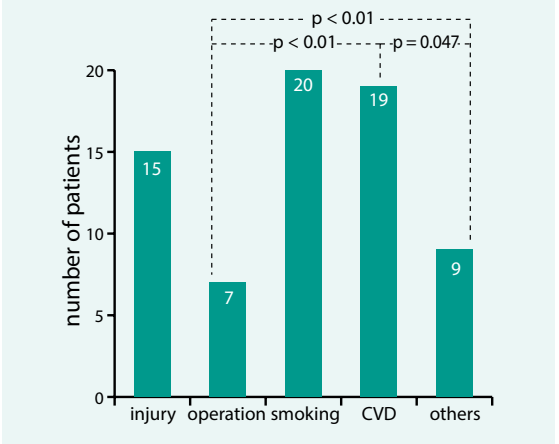
In summary, 2 274 patients with thrombotic events in the personal history and 303 control subjects were tested. The PTM was diagnosed in 157 patients (6.9 %) and 8 subjects (2.6 %) of the control group. According to the gender 92 (59 %) of patients were women, 65 (41 %) were men. In the control group, the ratio between men and women were proportional, see tab 1.

The average age of patients at the time of first thrombosis was 35.9 years and the median age was 34 years (interquartile range 18–64 years). The age distribution data are presented in graph 1, 2.

According to the PCR we discovered 156 heterozygotes and 1 homozygote in the group of patients. All subjects in control group were heterozygotes.

The most common clinical manifestation of PTM was the venous thrombosis – 123 events. In this case, the gender distribution was similar. The arterial thrombosis was presented less – 46 events. Interestingly, 1 patient had both, the arterial and venous thrombosis. The occurrence of PTM or PTM with FVL was relatively frequent in patients with arterial thrombosis, see tab. 1. The PTM was manifested by spontaneous abortions in 14 patients. Concomitant mutation of FVL gene was detected in 3 of these patients. Detailed analysis of data

**Graph 5. The presence of acquired thrombophilia risks among patients with PTM**




**Tab. 2. Geographical distribution of PTM in Europe**

| | VTE | H |
|---|---|---|
| Britain | 5 | 2,6 |
| Czech republic | 6 | 1,0–2,0 |
| France | 4,6 | 1,0–3,1 |
| Croatia | 8,0–8,3 | 2,5–4,0 |
| Italy | 4,3–15,9 | 1,0–2,3–5,7 |
| Poland | 6,5 | 1,8 |
| Sardinia | 16,5 | 4,8 |
| Slovenia | 5,8–11,3 | 3,1–4,8 |
| Serbia | 11,4 | 2,3–6,0 |
| Spain | 2,7–17,2 | 2,9–6,5 |
| Sweden | 7,1 | 1,8 |
| Turkey | 4,0–1,05 | 0,7–8,0 |

**H** – general healthy population % **VTE** – VTE patients %

related to clinical manifestation of PTM is presented in graph 3, 4.

In relation with the use of hormonal contraception, PTM was manifested as the thromboembolic event in a subgroup of 11 women. As the additional acquired risk of thrombosis in women, we observed the presence of smoking. 10 women had a personal smoking history. 9 of them had a personal history of venous thrombosis. The combination of oral contraceptive pill use and smoking was present in one patient. In this case, the clinical manifestation of PTM was venous thrombosis.

Injury, surgery, immobilization, smoking and cardiovascular disease (CVD) were the most frequent acquired risk factors for thrombosis in patients with PTM. Diabetes and cancer represent only a small percentage of acquired risk factors. We demonstrated statistical significance of smoking and CVD compared to another acquired risk factors (graph 5).

## Discussion

In conclusion, we tested 2 274 patients with thrombotic events in the personal history and 303 control subjects. The PTM was diagnosed in 157 patients (6.9 %) and in 8 controls (2.6 %). Using PCR we discovered 156 heterozygotes and 1 homozygote in the group of patients. All subjects in the control group were heterozygotes. Based on our results we can say that Slovakia is one of the European countries with high occurrence of the PTM in both groups. We believe it is caused by two main factors. On the one hand people are better informed about testing and prevention of thrombosis. On the other hand, a large group of medical specialists such as angiologists, neurologists, cardiologists, and gynaecologists refer their patients to a haematologist as any unprovoked episode of thrombosis that occurs in young adults < 50 years or any repeated thrombosis should be investigated for thrombophilia. Based on the above facts, the number of outpatient visits to haematologists has increased. Most of these patients are referred for differential diagnosis of the thromboembolic event or spontaneous abortion.

The geographical distribution of PTM in Europe is presented in tab. 2, [27–58]. In the presence of PTM, age and gender are independent risk factor for thrombosis [3]. In our study, there were 92 (59 %) women and 65 (41 %) men in the patient group. The PTM clinically manifested most often during the third and fourth decades of life. A large proportion of the female subjects in our sample can be explained by the fact that women of childbearing age are most often referred for the testing. Concerns about the use of OCs, spontaneous abortions, as well as their interest to find out the causes of thrombosis were the main characteristics of this age/gender group. Therefore, we can assume that the thrombophilia testing is indicated more often for females than males. Another part of the patients in this group was the subjects in whom the clinical manifestation of thrombosis could be explained by the presence of another congenital or acquired risk factors (FVL, injury, smoking, OCs, etc.).

Among Caucasian population the prevalence of congenital thrombophilia associated with venous thromboembolism is between 24 and 37 % compared to 10 % for the control group [60,61]. Thrombosis in the patients with congenital thrombophilia is often initiated by external stimuli, which disrupts the labile haemostatic balance and starts a vicious cycle of thromboembolic complications. Among acquired thrombophilia risk factors, we demonstrated statistically significant difference in the incidence of smoking and CVD. Spontaneous thrombosis occurs rarely (0.4 % cases) [62].

## Conclusions

Employing statistical analysis, we found relatively high prevalence of the PTM in both, the patients with

positive thrombosis history (6.9 %), as well as in the control group (2.6 %). In the Slovak patients with clinical signs of thrombophilia we have found the PTM to be a fairly frequent risk factor contributing particularly to the occurrence of venous and arterial thrombosis. Therefore, we think that PTM (heterozygous and homozygous variant) is a risk factor for arterial and venous thrombosis. In our sample there were only a few spontaneous abortions; nevertheless, they represent a significant clinical problem. Our study can confirm that the risk of thrombosis in carriers of PTM is independent of age and gender. The results showed relatively frequent presence of double carriership of the PTM and FVL.

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