Analysis of *PROS1*, *EPCR* and *PROC* single nucleotide polymorphisms in patients with myeloproliferative neoplasms

6th Kaunas / Lithuania International
Hematology / Oncology Colloquium
28 May 2021

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Objective

Patients with Philadelphia-negative (Ph-) myeloproliferative neoplasms: primary myelofibrosis (PMF), essential thrombocythemia (ET) and polycythemia vera (PV); often encounter thrombotic events and other disease complications. It was suggested that the genetic variants that are responsible for blood coagulation play causal role in the development of thrombosis.

In this work, we evaluated the effect of single nucleotide polymorphisms (SNPs) in *PROS1*, *EPCR*, *PROC* genes and the risk of developing thrombosis as well as clinical characteristics in patients with myeloproliferative disorders.

Methods

- Biological material was obtained from 88 patients who were diagnosed with ET, PV or PMF between 2000 and 2014 at the Department of Hematology of the Institute of Oncology, the Lithuanian University of Health Sciences, Kaunas, Lithuania.
- This study was approved by Kaunas Regional Ethics Committee for Biomedical Research (protocol no. BE-2-9). Patients provided written informed consent for the participation in the study.
- Medical information was collected such as the patient's age, sex, history of arterial or venous thrombosis, mean platelet volume and platelet count.
- Venous blood samples were collected in vacutainers with EDTA as anticoagulant. Samples were stored at -20°C until further processing. Genomic DNA was isolated from peripheral blood leukocytes using a commercially available DNA extraction kit, according to manufacturer's recommendations (Thermo Fisher Scientific Baltics, Vilnius, Lithuania).
- Genomic variants in all of the cases were detected by employing a polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method (Figures 1–4).
- The statistical analysis was done with *Statistical Package for the Social Sciences (SPSS) 20.0 for Windows*.

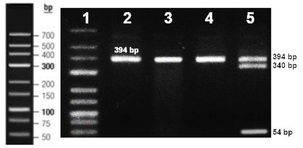

Figure 1. PROS1 g.66847T>C variant detection by PCR-RFLP. Electrophoretic patterns following AccI digestion. PCR products were 394 bp. Only amplicons with polymorphic allele were digested. The digestion products were separated by 2% agarose gel electrophoresis. Lane 1: DNA marker GeneRulerTM Low Range (Thermo Fisher Scientific); Lanes 2–4: TT genotype (394 bp); Lane 5: TC genotype (394, 340, 54 bp).

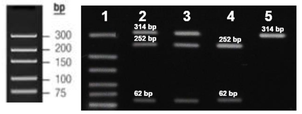

Figure 2. EPCR c.4678G>C variant detection by PCR-RFLP. Electrophoretic patterns following DdeI digestion. PCR products were 314 bp. Only amplicons with polymorphic allele were digested. The digestion products were separated by 2% agarose gel electrophoresis. Lane 1: DNA marker GeneRulerTM Ultra Low Range (Thermo Fisher Scientific)*; Lanes 2, 3: GC genotype (314, 252, 62 bp); Lane 4: CC genotype (252, 62 bp); Lane 5: GG genotype (314 bp).*

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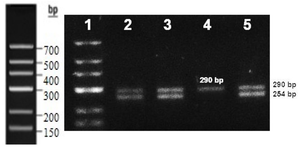

Figure 3. EPCR c.6936A>G variant detection by PCR-RFLP. Electrophoretic patterns following PstI digestion. PCR products were 290 bp. Only amplicons with polymorphic allele were digested. The digestion products were separated by 2% agarose gel electrophoresis. Lane 1: DNA marker GeneRulerTM Low Range (Thermo Fisher Scientific); Lanes 2, 3, 5: AG genotype (290, 254 bp); Lane 4: GG genotype (290 bp).

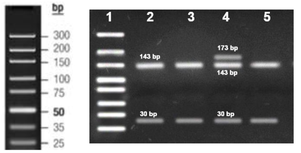

Figure 4. PROC c.565C>T variant detection by PCR-RFLP. Electrophoretic patterns following Hin6I digestion. PCR products were 173 bp. The digestion products were separated by 2% agarose gel electrophoresis. Lane 1: DNA marker GeneRulerTM Ultra Low Range (Thermo Fisher Scientific); Lanes 2, 3, 5: CC genotype (143, 30 bp); Lane 4: CT genotype (173, 143, 30 bp).

Results

- The patient group consisted of men (42%) and women (58%), mean age 64.2 years (standard deviation 14,436), range 27–87 years.
  Diagnosis was conformed as ET in 45 patients (51.1%), PV in 36 (40.9%) and PMF in seven (8%) patients of the studied group.
- The distribution of thrombotic events and clinical characteristics is summarized in Table 1.
- The genotypes of *PROS1 g.66847T>C, EPCR c.4678G>C, EPCR c.6936A>G and PROC c.565C>T* were under Hardy-Weinberg Equilibrium (Figure 5).
- ***PROS1* g.66847T>C.** According to logistic regression analysis, carriers of the *PROS1* 66847TC genotype may have a lower chance of developing venous thrombosis (Table 2).
- ***EPCR* c.4678G>C.** Carriers of 4678GC genotype are less susceptible to venous thrombosis development (Table 2).
- ***EPCR* c.6936A>G.** The heterozygous AG genotype may have a lower risk of developing venous or arterial thrombosis.
  Moreover, the 6936GG genotype was revealed to reduce the risk of venous thrombosis development (Table 2).
- ***PROC* c.565C>T.** Logistic regression analysis showed that there is a lower chance of developing venous thrombosis for carriers of the 565CT and 565TT genotypes.
  Occurrence of thrombosis overall is lower to those who have the 565TT genotype.
  The carriers of 565CT and 565TT genotypes had a lower chance of decreased mean platelet volume (Table 2).

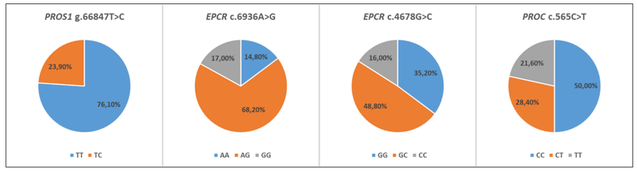

Figure 5. PROS1, EPCR and PROC polymorphisms genotype distribution.

Table 1. The distribution of clinical characteristics.

Characteristics	Patients (n=88)
Arterial thrombosis: n (%)	29 (33)
Venous thrombosis: n (%)	10 (11.4)
Incidence of thrombosis: n (%)	39 (44)
Decreased mean platelet volume: n (%)	22 (25)
Normal mean platelet volume: n (%)	22 (25)
Increased mean platelet volume: n (%)	1 (1.1)

Table 2. Odds ratio for the association of PROS1, EPCR, PROC polymorphisms with thrombosis risk and clinical characteristics.

Polymorphism	Genotype	Clinical characteristic	B	OR	*p* value	95% CI-OR
PROS1 g.66847T>C	TC	Venous thrombosis	-2.773	0.063	0.007	0.008 – 0.471
EPCR c.4678G>C	GC	Venous thrombosis	-1.488	0.226	<0.001	0.099 – 0.513
EPCR c.6936A>G	AG	Venous thrombosis	-1.768	0.171	<0.001	0.077 – 0.381
	GG		-2.485	0.083	0.017	0.011 – 0.641
	AG	Arterial thrombosis	-0.663	0.515	0.026	0.287 – 0.925
PROC c.565C>T	CT	Venous thrombosis	-1.846	0.158	0.003	0.047 – 0.534
	TT		-2.078	0.067	0.009	0.009 – 0.505
	TT	Incidence of thrombosis	-1.030	0.357	0.048	0.129 – 0.992
	CT	Decreased mean platelet volume	-0.944	0.389	0.034	0.162 – 0.931
	TT		-1.322	0.267	0.019	0.089 – 0.803

Conclusions

It can be concluded that *PROS1*, *EPCR* and *PROC* single nucleotide polymorphisms may be associated with thrombotic events. However, more research is needed, since there are many conflicting results published regarding the complexity of the possible interactions between these genetic variants and predisposition to thrombotic events.

Key words

Myeloproliferative neoplasms; thrombosis; *PROS1*; *EPCR*; *PROC*; polymorphisms.