

# Glycoprotein VI Gene Variants Affect Pregnancy Loss in Patients With Platelet Hyperaggregability

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Juraj Sokol, MD, PhD<sup>1</sup>, Maria Skerenova, MA, PhD<sup>2</sup>,  
Kamil Biringer, MD, PhD<sup>3</sup>, Tomas Simurda, MD, PhD<sup>1</sup>,  
Peter Kubisz, MD, DSc<sup>1</sup>, and Jan Stasko, MD, PhD<sup>1</sup>

## Abstract

The aim of our study was to evaluate GP6 gene in patients with sticky platelet syndrome (SPS) and fetal loss. Platelet aggregability was tested with platelet-rich plasma using PACKS-4 aggregometer (Helena Laboratories). High-resolution melting analysis on LightCycler 480 II (Roche Diagnostics) was used for single-nucleotide polymorphism (SNP) genotyping. We examined 64 patients with SPS and 54 control participants. We found significantly higher occurrence of 5 SNPs in patients with SPS versus controls (rs1671152, rs1654433, rs1613662, rs1654416, and rs2304167). Moreover, the haplotype analysis showed a significantly higher occurrence of 7 haplotypes in patients with SPS compared to controls (acgg and aagg in GP6\_5reg haplotype; ccgt in GP6\_3reg haplotype; gg and ta in GP6\_REG haplotype; SKTH and PEAN in GP6\_PEAN haplotype). Our results, especially higher occurrence of 4 nonsynonymous variants within the coding region, support the idea that GP6 polymorphisms are associated with the platelet hyperaggregability accompanied by fetal loss.

## Keywords

hyperaggregability, fetal loss, glycoprotein VI, single-nucleotide polymorphism

## Introduction

Worldwide, obstetric complications (recurrent pregnancy loss, fetal growth retardation, preeclampsia, and placental abruption) are a serious health problem associated with morbidity and mortality. These complications occur in between 1% and 5% of pregnant women.<sup>1-4</sup>

Sticky platelet syndrome (SPS) is a hereditary platelet disorder. Sticky platelet syndrome is characterized by increased in vitro platelet hyperreactivity to adenosine diphosphate (ADP) and/or epinephrine (EPI).<sup>5,6</sup> Patients with SPS present with arterial and/or venous thrombosis and fetal loss.<sup>5-12</sup> Although the first patient with SPS was reported in 1983,<sup>13</sup> many physicians remain unfamiliar with this platelet defect and do not consider SPS when screening for thrombophilic risk factors. The diagnosis and classification of SPS relies on platelet aggregometry tests employing appropriate dilutions of 2 platelet aggregation inducers: ADP and EPI.<sup>5,6,12</sup>

Since both genders are affected, SPS has a clear autosomal pattern of inheritance, although the exact genetic cause has not been identified yet. It has been suggested that the defects of the platelet membrane glycoproteins or intracellular signal pathways involved in platelet activation and aggregation are responsible for the disorder.<sup>6,10</sup>

Glycoprotein VI (GPVI) is a 60- to 65-kDa transmembrane protein consisting of 2 immunoglobulin-like domains, a highly glycosylated stem, a 19-amino acid transmembrane domain and a 51-amino acid cytoplasmic tail.<sup>14-16</sup> The GPVI was shown by several in vitro and in vivo studies to be essential for activation of the integrin for stable adhesion and subsequent signal transduction (via activation of phosphatidylinositol-3-kinase and phospholipase C $\gamma$ 2) that leads to granule release, activation of the GPIIb/IIIa via inside out signaling, and platelet aggregation.<sup>15</sup> Glycoprotein VI is a product of the GP6

<sup>1</sup> Department of Hematology and Transfusion Medicine, National Center of Hemostasis and Thrombosis, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

<sup>2</sup> Department of Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

<sup>3</sup> Department of Gynecology and Obstetrics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

## Corresponding Author:

Juraj Sokol, Department of Hematology and Transfusion Medicine, National Center of Hemostasis and Thrombosis, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Kollarova 2, Martin 03659, Slovakia.  
Email: juraj.sokol@me.com



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**Table 1.** Platelet Aggregation Concentration and Values.

	Platelet Aggregation After Activation With					
	ADP			EPI		
Concentration, $\mu\text{M}$	0.58	1.17	2.34	0.55	1.1	11.0
Normal range, % <sup>a</sup>	0.0-12.0	2.0-36.0	7.5-55.0	9.0-20.0	15.0-27.0	39.0-80.0

Abbreviations: ADP, adenosine diphosphate; EPI, epinephrine.

<sup>a</sup>Only informative, can be different for each laboratory.

**Table 2.** Characteristics of the Selected SNPs.

Ref SNP ID Number	Position on Chromosome 19	Position Within GP6 Gene	SNP Sequence	Major/Minor Allele
rs1671152	55014977	Exonic	CCCGT(G/T)TGATT	G/T
rs1671215	55041651	3' regulatory region	TGTAA(A/C)TGCA	A/C
rs12981732	55013007	Intronic	GCAGC(C/T)GAGAA	C/T
rs4281840	55005440	5' regulatory region	GGAAG(C/A)GTTTT	C/A
rs8113032	55042770	Intronic	CGCAC(G/A)TGTTT	G/A
rs1654433	55038944	5' regulatory region	AATTG(G/A)ACTG	G/A
rs10417943	55013969	Exonic	TGAGA(G/A)GGGTG	G/A
rs10418743	55042000	5' regulatory region	AGAAC(G/A)CTTCC	G/A
rs1654410	55013445	3' regulatory region	TCTGT(C/T)TTCTG	C/T
rs11669150	55024604	Intronic	TGAGA(T/C)GTTTG	T/C
rs1613662	55025227	Exonic	TACCG(A/G)GGAAG	A/G
rs12610286	55030031	Intronic	TTAAC(A/G)TATTT	A/G
rs1654431	55038390	5' regulatory region	TAGCC(G/A)GCTCC	G/A
rs1654416	55018667	Exonic	GACTT(T/C)GTTTGT	T/C
rs2304167	55015713	Exonic	ACTGG(C/T)GGTGA	C/T

Abbreviations: A, adenine; C, cytosine; G, guanine; SNP, single-nucleotide polymorphism, T, thymine.

gene, localized on chromosome 19 (19q13.4).<sup>17</sup> Since the identification and analysis of the GP6 gene in the 1990s, its numerous single-nucleotide polymorphisms (SNPs) have been identified.<sup>18</sup>

The aim of this 12-year prospective study was to evaluate the variability of GP6 gene in a group of patients with SPS manifested as miscarriage compared to control participants and to determine the relationship between selected regions of GP6 gene and SPS.

## Patients and Methods

### Study Population and Inclusion/Exclusion Criteria

The local ethical committee of the Jessenius Faculty of Medicine in Martin approved the study (Number EK950/2011). All study participants agreed to participate in the project and signed a written informed consent in accordance with the Declaration of Helsinki, including healthy female blood donors.

All patients were enrolled into this study between March 2005 and September 2017. Patients were initially examined and tested at the Department of Hematology and Transfusiology in Martin University Hospital. After other causes of spontaneous abortion (hormonal, endocrine, genetic, and anatomical) were excluded, the patients were referred to undergo the thrombophilia screening as a part of a spontaneous

abortion differential diagnosis. Patients with a verified spontaneous abortion and SPS were asked to participate in the genotype testing. Thus, all patients fulfilled the following inclusion criteria: at least 1 spontaneous abortion and confirmation of SPS according to the criteria of Mammen,<sup>7</sup> documented at least twice by 2 distinct assessments with an interval of at least 1 month. A total number of patients with 1 to 4 spontaneous abortions were 64: 11 (17.2%), 40 (62.5%), 12 (18.8%), and 1 (1.5%), respectively. The occurrence of other thromboembolic states (such as factor V Leiden, prothrombin G20210A, methylenetetrahydrofolate reductase C677T mutations, protein C, protein S, and antithrombin deficiencies, and the presence of antiphospholipid syndrome) was considered as a reason for an exclusion of patients. Furthermore, 54 randomly chosen healthy female blood donors from the same region of Žilina, the northwestern part of Slovakia, were involved as control participants. All control participants were Caucasians of European origin, with negative personal and family history of the venous thromboembolism and normal platelet aggregability after stimulation by an EPI and/or ADP agonist according to the method by Mammen.<sup>7</sup> For both groups, exclusion criteria included occurrence of thromboembolic events, active or chronic disease, chronic medication, current pregnancy, and/or smoking.

All blood samples in both study groups were taken outside the menstrual period or other active bleeding, at least 6 months

**Table 3.** The Gestation Week of Spontaneous Abortion.

Patient Number	First Fetal Loss	Second Fetal Loss	Third Fetal Loss	Fourth Fetal Loss
1	10	11	7	-
2	8	8	10	-
3	12	9	-	-
4	28	18	-	-
5	7	8	-	-
6	5	7	-	-
7	14	-	-	-
8	11	-	-	-
9	20	20	-	-
10	7	7	8	-
11	10	20	-	-
12	6	12	-	-
13	12	12	17	-
14	4	7	9	-
15	7	12	-	-
16	9	10	-	-
17	29	-	-	-
18	10	11	-	-
19	11	-	-	-
20	27	-	-	-
21	5	5	-	-
22	20	22	-	-
23	20	20	-	-
24	12	12	13	-
25	10	12	-	-
26	15	13	-	-
27	7	8	13	-
28	8	9	-	-
29	15	13	-	-
30	16	20	-	-
31	9	10	11	11
32	8	8	-	-
33	7	8	-	-
34	8	8	-	-
35	9	10	-	-
36	12	11	-	-
37	20	-	-	-
38	9	10	11	-
39	9	-	-	-
40	10	11	-	-
41	13	-	-	-
42	11	13	13	-
43	12	11	-	-
44	12	-	-	-
45	10	13	-	-
46	12	10	14	-
47	13	8	-	-
48	10	12	-	-
49	15	12	16	-
50	12	12	-	-
51	16	12	-	-
52	13	12	-	-
53	13	10	13	-
54	13	18	-	-
55	14	12	-	-
56	14	-	-	-
57	13	10	-	-

(continued)

**Table 3.** (continued)

Patient Number	First Fetal Loss	Second Fetal Loss	Third Fetal Loss	Fourth Fetal Loss
58	10	11	-	-
59	13	12	-	-
60	14	13	-	-
61	10	8	-	-
62	13	-	-	-
63	12	12	-	-
64	14	12	-	-

after a spontaneous abortion. Inflammatory process was excluded by measuring C-reactive protein level (normal value < 5.0 mg/L) and normal white blood cell count (normal range =  $4-10 \times 10^9/L$ ).

### Diagnostics of SPS

The antecubital venous blood was collected into the tubes of 3.2% buffered sodium citrate (anticoagulant–blood ratio, 1:9) to assess the platelet aggregation. The samples were processed and analyzed within 2 hours after sampling. Platelet aggregability was tested with platelet-rich plasma (PRP) using platelet aggregometry (PACKS-4 aggregometer; Helena Laboratories, Texas, USA) according to Mammen.<sup>7</sup> Each sample was tested with 3 low concentrations of ADP (2.34, 1.17, and 0.58  $\mu\text{mol/L}$ ) and EPI (11.0, 1.1, and 0.55  $\mu\text{mol/L}$ ), see Table 1.

Citrated blood samples obtained as described above were centrifuged to prepare PRP and platelet-poor plasma (PPP). To prepare PRP, whole blood tubes were centrifuged at 170 g to 200g for 10 minutes in a swing-out rotor. Autologous PPP was prepared by centrifugation at a minimum of 1500g for at least 15 minutes. At the end of the centrifugation steps, a plastic pipette was used to separate the top two-thirds of PRP or PPP. The PRP was then left for at least 30 minutes prior to testing. It is important that samples are preincubated for at least 5 minutes at 37°C prior to assay to obtain stable baseline traces. The appropriate agonists were then added directly to the PRP. The aggregation tracing was observed for at least 10 minutes.

### DNA Analysis

Antecubital venous blood used for DNA analysis was collected into tubes containing 5.4 mg K2EDTA (spray coated). Samples were processed within 2 hours of collection and stored, if necessary, at -20°C. DNA was extracted from peripheral blood leukocytes. Isolation of genomic DNA from whole blood was performed using an SiMax Genomic DNA Extraction kit (SBS Genetech Co, Ltd, Beijing, China) according to the manufacturer's instructions. High-resolution melting analysis on Light-Cycler 480 II (Roche Diagnostics, Mannheim, Germany) was used for SNP genotyping. The selection and designing of primer sequences (Table 2) was performed by Primer3 software.<sup>18</sup>

**Table 4.** Frequency of Alleles of the GP6 Regulatory Regions SNP in Patients With SPS and Control Individuals.

Ref SNP	Freq. (SPS)	Freq. (Control)	Fisher HWE P (SPS)	Fisher HWE P (Control)	Fisher Exact P Allelic Association	OR Minor Allele	95% CI
<b>rs1671152</b>	<b>0.203</b>	<b>0.046</b>	<b>1.000</b>	<b>1.000</b>	<b>.000</b>	<b>5.251</b>	<b>1.940-14.20</b>
rs1671215	0.328	0.213	.398	1.000	.057	1.805	1.000-3.256
rs12981732	0.227	0.250	.492	1.000	.759	0.879	0.481-1.602
rs4281840	0.320	0.343	.565	.231	.782	0.904	0.524-1.557
rs8113032	0.422	0.472	1.000	.411	.511	0.816	0.487-1.365
<b>rs1654433</b>	<b>0.195</b>	<b>0.037</b>	<b>1.000</b>	<b>1.000</b>	<b>.000</b>	<b>6.311</b>	<b>2.121-18.77</b>
rs10417943	0.008	0.000	1.000	1.000	1.000	-	-
rs10418743	0.125	0.130	1.000	1.000	1.000	0.959	0.445-2.067
rs1654410	0.438	0.435	.617	.588	1.000	1.009	0.602-1.691
rs11669150	0.222	0.269	1.000	1.000	.447	0.778	0.428-1.415
<b>rs1613662</b>	<b>0.180</b>	<b>0.065</b>	<b>1.000</b>	<b>1.000</b>	<b>.010</b>	<b>3.161</b>	<b>1.299-7.689</b>
rs12610286	0.258	0.343	1.000	.231	.198	0.667	0.380-1.168
rs1654431	0.500	0.444	.802	.789	.434	1.250	0.747-2.089
<b>rs1654416</b>	<b>0.188</b>	<b>0.046</b>	<b>.678</b>	<b>1.000</b>	<b>.001</b>	<b>4.754</b>	<b>1.746-12.93</b>
<b>rs2304167</b>	<b>0.188</b>	<b>0.046</b>	<b>.678</b>	<b>1.000</b>	<b>.001</b>	<b>4.754</b>	<b>1.746-12.93</b>

Abbreviations: CI, confidence interval, freq., frequency, HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism; SPS, sticky platelet syndrome.

### GP6 Gene Analysis

We selected representative SNPs in GP6 region (rs1671152, rs1671215, rs12981732, rs4281840, rs8113032, rs1654433, rs10417943, rs10418743, rs1654410, rs11669150, rs1613662, rs12610286, rs1654431, rs1654416, and rs2304167). For the selection of tag SNP, data from the International HapMap Project<sup>19</sup> and an adopted algorithm implemented in Haploview 4.2 were used.<sup>20</sup> The parameters for Haploview 4.2 were Hapmap data release 27 (phase II + III) on NCBI B36 assembly, dbSNP b126, chromosome 19, 3' end regions 60 206 to 60219 kb and 5' end regions 60 240 to 60 247 kb, analysis panel CEU,  $r = 0.8$ , minor allele frequency (MAF) > 0.01, pair wise tagging. The basic characteristics of analyzed SNP including nucleotide sequence is given in Table 2 and their further details are described elsewhere.<sup>21</sup>

### Statistics

We used IBM SPSS for Windows 16.0 (Statistical Package for the Social Sciences, version 16.0; SPSS Inc) for statistical analysis. The haplotype association analysis was performed using SNP and variation Suite 7 (SNP & Variation Suite v7.6.11; Golden Helix, Bozeman, Montana). The Fisher exact test was used to estimate the significance of deviation from Hardy-Weinberg equilibrium (HWE) and to execute basic allelic association.  $P$  values less than .05 were considered statistically significant. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess risk. A haplotype frequency was estimated using expectation-maximization (EM) algorithm.

### Results

We examined 64 patients having SPS with the age of  $35.3 \pm 6.7$  years and 54 healthy control participants with the age of

$42.54 \pm 14.1$  years; for the gestation week of spontaneous abortion, see in Table 3.

In sum, we examined 15 SNPs. Five were localized in the coding region of GP6 gene. Four of them were nonsynonymous missense variations in exon 5 (rs1613662), 6 (rs1654416), 7 (rs2304167), and 8 (rs1671152). One SNP was synonymous variant in exon 8 (rs10417943). Three selected polymorphisms were recognized in intronic regions (rs12981732, rs11669150, and rs12610286). We had also examined SNPs in GP6 regulatory regions on 5' and 3' ends (3' end: rs1671215 and rs1654410; 5' end: rs4281840, rs1654433, rs10418743, and rs1654431).

All mentioned nonsynonymous variations were tested for the deviation from HWE with significant findings (rs1671152: 0.203 vs 0.046, OR: 5.151, CI: 1.94-14.2; rs1613662: 0.18 vs 0.065, OR: 3.161, CI: 1.299-7.689; rs1654416: 0.188 vs 0.046, OR: 4.754, CI: 1.746-12.93; rs2304167: 0.188 vs 0.046, OR: 4.754, CI: 1.746-12.93). In addition, we found that 1 polymorphism in 5' regulatory region of GP6 gene was significantly more frequent in patients with SPS and history of fetal loss compared to healthy controls (rs1654433: 0.195 vs 0.037, OR: 6.311, CI: 2.121-18.77), see Table 4. The very low frequency of these SNPs analyzed in the healthy control may indicate that these mutations are localized in conservative regions of the gene and their substitution may lead to abnormal functional protein.

The haplotype analysis showed a significantly higher occurrence of 7 haplotypes (acgg: 0.138 vs 0.036, OR: 4.262, CI: 1.379, aagg: 0.039 vs 0.000 in GP6\_5reg haplotype; ccgt: 0.175 vs 0.039, OR: 5.242, CI: 1.79 in GP6\_3reg haplotype; gg: 0.78 vs 0.926, OR: 0.284, CI: 0.124; ta: 0.179 vs 0.009, OR: 23.439, CI: 3.085 in GP6\_REG haplotype and SKTH: 0.789 vs 0.935, OR: 0.192, CI: 0.071; PEAN: 0.18 vs 0.046, OR: 4.467, CI: 1.635 in GP6\_PEAN haplotype), see Table 5. In case we combine haplotype results with SNP frequency analysis, we find

**Table 5.** Haplotype Frequencies in Patients With SPS and Control Individuals.<sup>a</sup>

Haplotype		Frequency (SPS)	Frequency (Control)	P Value	OR	CI (95%)
GP6_H5	TTAAA	0.213	0.177	.420	1.309	0.680
	CCAAG	0.160	0.196	.543	0.812	0.414
	TTAGA	0.143	0.215	.178	0.628	0.318
	TTAAG	0.140	0.125	.673	1.178	0.550
	CTAGA	0.086	0.091	.943	0.968	0.391
	CTGAG	0.115	0.050	.064	2.559	0.919
	CCAAA	0.036	0.058	.442	0.617	0.178
	TTGAG	0.036	0.012	.229	3.142	0.442
	CTAAG	0.014	0.025	.543	0.553	0.080
	TCAAA	0.013	0.014	.985	0.979	0.108
	TTAGG	0.004	0.021	.251	0.199	0.009
	CTAGG	0.009	0.014	.747	0.672	0.060
	GP6_5reg	gaga	0.378	0.440	.336	0.774
gagg		0.149	0.223	.147	0.613	0.315
gcgg		0.152	0.171	.696	0.871	0.435
gaag		0.099	0.097	.969	1.017	0.430
<b>acgg</b>		<b>0.138</b>	<b>0.036</b>	<b>.007</b>	<b>4.262</b>	<b>1.379</b>
<b>aagg</b>		<b>0.039</b>	<b>0.000</b>	<b>.037</b>	-	-
gaaa		0.007	0.028	.204	0.237	0.021
gcaa		0.019	0.004	.295	4.817	0.188
acga		0.018	0.001	.188	20.743	0.033
GP6_3reg		ccgg	0.445	0.541	.159	0.691
	atgg	0.174	0.172	.949	1.023	0.520
	acgg	0.146	0.170	.629	0.841	0.417
	<b>ccgt</b>	<b>0.175</b>	<b>0.039</b>	<b>.001</b>	<b>5.242</b>	<b>1.790</b>
	ctgg	0.032	0.071	.175	0.435	0.127
	ctgt	0.021	0.007	.382	2.967	0.230
GP6_REG	<b>gg</b>	<b>0.780</b>	<b>0.926</b>	<b>.002</b>	<b>0.284</b>	<b>0.124</b>
	<b>ta</b>	<b>0.179</b>	<b>0.009</b>	<b>.000</b>	<b>23.439</b>	<b>3.085</b>
	tg	0.025	0.037	.576	0.654	0.146
	ga	0.017	0.028	.562	0.595	0.101
GP6_PEAN	<b>SKTH</b>	<b>0.789</b>	<b>0.935</b>	<b>.000</b>	<b>0.192</b>	<b>0.071</b>
	<b>PEAN</b>	<b>0.180</b>	<b>0.046</b>	<b>.002</b>	<b>4.467</b>	<b>1.635</b>
	SKTN	0.023	0.000	.111	-	-

Abbreviations: SNP, single-nucleotide polymorphism, SPS, sticky platelet syndrome.

<sup>a</sup>GP6\_H5 haplotype (rs1654410, rs11669150, rs1613662, rs12610286, and rs1654431), GP6\_5reg haplotype (rs1654433, rs1671215, rs10418743, and rs8113032), GP6\_3reg haplotype (rs4281840, rs12981732, and rs1671152), GP6\_REG haplotype (rs1671152 and rs1654433), and GP6\_PEAN haplotype (rs1671152, rs2304167, rs1654416, and rs1613662).

that the most interesting results are results of GP6\_PEAN haplotype. Using EM algorithm, the estimated minor haplotypes constructed from nonsynonymous variants (rs1671152, rs2304167, rs1654416, and rs1613662) with predicted protein PEAN and SKTH were significantly associated with the given SPS phenotype accompanied by fetal loss. Patients with PEAN variant of GPVI protein have 4.5 increase in risk of fetal loss versus healthy controls. In contrast, patients with SKTH variant are protected from fetal loss compared to controls (OR = 0.192). Patients with SPS and ccgt haplotype (OR = 5.242), which was constructed from 4 SNPs (rs1654433, rs1671215, rs10418743, and rs8113032), have the highest risk of fetal loss.

## Discussion

Pregnant women may experience a variety of adverse obstetric events, such as preeclampsia, placental abruption, fetal growth retardation, and pregnancy loss, and these may be related to alterations in placental perfusion. The low pressure and turbulent flow pattern of circulation at the placenta, along with changes in hypercoagulability during this period, may also predispose women to thrombosis. It is possible that some hypercoagulable states may predispose individuals to arterial thrombosis, which can then result in uteroplacental thrombosis and obstetric complications. However, the exact mechanism is not known.<sup>22</sup>

In 2012, we pointed out that 3 selected SNPs (rs1671153, rs1654419, and rs1613662) of the GP6 gene occur more frequently in patients having SPS with a history of miscarriage. Unfortunately, 2 of these SNPs were localized in the intron region (rs1671153 and rs1654419). Therefore, the changes most likely to not occur on the GPVI protein.<sup>23</sup>

Kotuličová et al reported about the same SNPs. But her research was focused on patients having SPS with deep vein thrombosis. She described that these selected GP6 gene polymorphisms are an independent risk factor for deep vein thrombosis in patients with platelet hyperaggregability.<sup>24</sup>

In this work, we focused on regulatory regions, exons and introns, of the GP6 gene in patients having SPS with a history of fetal loss. We identified 4 nonsynonymous variants within the coding region, which were significantly associated with the SPS phenotype accompanied by fetal loss (rs1671152: 4.4 times more often in patients having SPS with a history of fetal loss compared to healthy volunteers; rs1613662: 2.8 times; rs1654416 and rs2304167: 4.1 times). The SNP rs1613662 of GPVI leads to the substitution of serine 219 by proline and has been associated with coronary thrombus formation.<sup>25</sup> The remaining nonsynonymous variants (rs1654416, rs2304167, and rs1671152) are associated with amino acid substitutions: Lys237Glu, Thr249Ala, and His322Asn.<sup>26</sup> We and others showed independently that these variants are in linkage disequilibrium and create minor haplotypes at protein level with protein residues PEAN (in successive order: rs1613662, rs1654416, rs2304167, and rs1671152).<sup>27</sup> Three of these SNPs (rs1613662, rs1654416, and rs2304167) are localized in the extracellular topological protein domain.<sup>28</sup> In a functional study, PEALN haplotype revealed a significant difference in membrane expression of GPVI.<sup>27</sup> The rs1671152 variant causes missense cytoplasmic mutation. It is assumed that PEALN haplotype has significant effect on GPVI-mediated signal transduction.<sup>29</sup> We also showed that the normal SKTH haplotype has protective effect against platelet hyperaggregability.

In addition, we found that 1 polymorphism in 5' regulatory region of GP6 gene was significantly more frequent in patients with SPS and a history of fetal loss compared to healthy controls.

There were several limitations to our study, including the limited number of patients with abortion and a possible bias in

patient selection. Sticky platelet syndrome in the Slovak population in association with a history of miscarriage is extremely rare. Despite this, this study represents the largest sample of patients having SPS with a history of miscarriage. As far as the SPS diagnostic process is concerned, there are also several limitations from a methodological standpoint. Platelet aggregability is greatly affected by preanalytical issues, and therefore, interpretation of platelet hyperaggregability is potentially accordingly adversely influenced. Moreover, functional studies of ligands and pathways of GPVI which are going to analyze different isoforms have to be performed.

## Conclusions

Our results, especially higher occurrence of 4 nonsynonymous variants within the coding region, support the idea that GP6 gene polymorphisms are associated with the platelet hyperaggregability—a possible cause of fetal loss. This study has extended our knowledge of genetic variability of GP6 gene in platelet function.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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