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GP6 Haplotype of Missense Variants is Associated with Sticky Platelet Syndrome Manifested by Fetal Loss

Mária Škereňová, MSc[1], Juraj Sokol, MD, PhD[2], Kamil Biringer, MD, PhD[3], Jela Ivanková, MSc[2], Ján Staško, MD, PhD[2,4], Peter Kubisz, MD, PhD[2,4], and Zora Lasabová, MSc, PhD[5,6]

Abstract

Disequilibrium of hemostasis is central to the pathogenesis of all thromboses, and platelets are essential for primary hemostasis. The platelet membrane glycoprotein receptor is involved in the clot formation in blood; therefore, the changes in related genes could impair platelet aggregation in patients with sticky platelet syndrome (SPS). Patients with SPS who experienced fetal loss were shown to harbor a risk haplotype at *GP6* locus. The aim of the study was to examine the genetic linkage of this selected risk haplotype with single nucleotide variations (SNVs) in the coding sequence of the *GP6* gene in order to identify possible functional SNVs in association with SPS and fetal loss. A total of 37 patients with SPS manifested fetal loss, and 42 healthy controls were enrolled in the study. The SPS was diagnosed with platelet aggregometry. The SNVs were determined by dideoxy sequencing and high-resolution melting analysis. The missense variations were detected in patients with risk haplotype only. The association analysis showed association of the minor alleles with the SPS manifested by fetal loss as follows—rs16711152 (odds ratio [OR]: 4.667, 95% confidence interval [CI]: 1.462-14.89, $P = .006$), rs2304167 (OR: 5.085, 95% CI: 1.605-16.10, $P = .003$), and rs1654416 (OR: 5.085, 95% CI: 1.605-16.10, $P = .003$). Using the Expectation-Maximization (EM) algorithm, the estimated minor haplotype with predicted protein residue PEAN was significantly associated with the given phenotype (OR: 4.746, 95% CI: 1.486-15.15, $P = .005$). We have shown that haplotype PEAN associated with SPS and manifested by fetal loss and suggest that the mechanism involved in the action of GPVI has significant effect on GPVI-mediated signal transduction through Syk-phosphorylation.

Keywords

sticky platelet syndrome, fetal loss, *GP6*, haplotype, missense variation

Introduction

Thrombotic disorders in humans are the most common causes of morbidity and mortality. Disequilibrium of hemostasis is central to the pathogenesis of all thromboses, and platelets are essential for primary hemostasis. Using platelet aggregometry, it was demonstrated that the enhanced maximal platelet aggregation is a contributing factor for arterial and venous thromboses, naming this condition sticky platelet syndrome (SPS). The in vitro platelet aggregation after activation by adenosine diphosphate (ADP) and/or epinephrine (EPI) is characteristic for SPS type I, hyperaggregation by EPI alone defined the SPS type II, and hyperaggregation by ADP alone triggers hyperaggregation in SPS type III.[1-3] The SPS has been established as a factor playing a role in the development of angina pectoris, acute myocardial infarction, transient cerebral ischemic attacks, stroke, ischemic optic neuropathy, and venous thrombosis; however not all carriers of the syndrome experience clinical symptoms.[1,4] Furthermore, this syndrome has been described also in women with recurrent fetal loss without a

[1] Department of Clinical Biochemistry, Jessenius Faculty of Medicine and University Hospital in Martin, Comenius University in Bratislava, Martin, Slovakia
[2] Department of Hematology and Blood Transfusion, National Center of Hemostasis and Thrombosis, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia
[3] Department of Gynecology and Obstetrics, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia
[4] Biomedical Center Martin, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Division Molecular Medicine, Martin, Slovakia
[5] Department of Molecular Biology, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia
[6] Biomedical Center Martin, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Division Oncology, Martin, Slovakia

Corresponding Author:
Zora Lasabová, Division Oncology, Biomedical Center Martin, Jessenius Faculty of Medicine, Comenius University in Bratislava, Malá hora 4C, SK-03601 Martin, Slovakia.
Email: lasabova@jfmed.uniba.sk

personal history of thrombosis. Although the association between inherited thrombophilia and venous thromboembolism is well established, a definitive link between inherited thrombophilia and adverse pregnancy outcomes has not been made yet.[5,6] In a report of 351 women undergoing evaluation for recurrent fetal loss, SPS was identified in 64 of them and was the most prevalent hemostatic defect.[7] It was shown that SPS segregates in families, although the genetic basis underlying this syndrome was not revealed yet.[8] The etiology of SPS is still unknown, but the published epidemiological data and the results from segregation studies in families sustain suggestion that the glycoprotein (GP) receptors on the platelet surface membrane could play an important role in the pathogenesis of SPS. The membrane GPs are involved in the clot formation in blood; therefore, the changes in related genes could impair platelet aggregation in SPS. It was also reported that certain mutation can modulate the risk of thromboembolism, although all studies failed to prove that a single genetic defect is responsible for SPS.[9,10] In a genome-wide meta-analysis, 2.5 million single-nucleotide polymorphism (SNPs) with platelet aggregation response to 3 agonists (ADP, EPI, and collagen) were tested in 2 European ancestry cohorts and identified 7 loci with platelet aggregation, 1 among them near/in the *GP6* gene.[11] Several studies including those of us have found out that single nucleotide polymorphisms of certain GPs such as GPIIIa, Gas6, or GPVI are in association with SPS.[10,12-14] Moreover, the certain SNPs in the *GP6* gene were shown to be associated with miscarriages in patients with SPS type I or II, and haplotypes were identified, which have higher occurrence in patients with SPS experiencing fetal loss. In our previous case–control study, we were able to identify the risk haplotype "CTGAG" consisting of rs1654410, rs1669150, rs1613662, rs12610286, and rs1654431 with odds ratio (OR) comprising 3.568 in patients with SPS experiencing fetal loss.[15,16]

In this study, we sequenced the whole protein coding region of the *GP6* gene. The aim of this study was to examine the genetic linkage of the risk haplotypes "CTGAG" reported previously [15]with SNVs in the coding sequence of the *GP6* gene in order to identify possible functional SNVs in association with SPS and fetal loss.

Patients and Methods

Patients

Patients with verified miscarriage (at least 1) were enrolled in the study and were referred to undergo thrombophilia screening as a part of differential diagnosis for spontaneous abortion examined at the Department of Hematology and Transfusiology. In case of confirmation of SPS according the criteria of Mammen (see below), they were asked to participate in the study. The occurrence of other thromboembolic events such as venous thromboembolisms, myocardial infarction, and cerebrovascular thrombosis was not considered as a reason for the exclusion of the patients. A total of 37 patients with SPS and 42 female healthy blood donors with negative personal and family history and normal platelet aggregability were enrolled in the study. The study was approved by the ethical committee and each participant signed the informed consent to be able take a part in the study. We screened patients for thrombophilia after exclusion of anatomical, chromosomal, and endocrinological disorders. The test for thrombophilia was done at least 6 months after miscarriage. A total number of patients with 1 to 4 spontaneous abortions were 6 (16.2%), 23 (62.16%), 7 (18.91%), and 1 (2.70%), respectively. Detailed characteristics of patients, number and times, and gestational week of abortion are summarized in the table, which is attached to the manuscript as Supplemental Table 1. In addition, we performed subgroup analysis based on the number of miscarriage. We had excluded patients with only 1 spontaneous abortion. This reduced group of patients was called "cases 6."

The aggregometry testing was performed on patients without antiplatelet therapy. The administration of acetylsalicylic acid or ADP inhibitor treatment were interrupted for more than 7 days before testing, and the use of other drugs with possible effect on platelet activity was omitted. The patients were without the occurrence of acute thromboembolic events ($\geq$3-5 months). All 9 patients with SPS having the risk haplotype "CTGAG" and 9 participants with SPS not having the risk haplotype who experienced fetal loss estimated in a previous study[15] were selected for the dideoxy sequencing.

Diagnosis of SPS by the Aggregometry by Mammen

The antecubital venous blood was collected with sodium citrate to assess the platelet aggregation, and the samples were processed and analyzed within 2 hours after sampling according to Mammen as described by us previously.[15] Briefly, each sample of platelet-rich plasma was tested with 3 low concentration of ADP (2.34 mmol/L, 1.17 mmol/L, and 0.58 mmol/L) and EPI (11.00 mmol/L, 1.1 mmol/L, and 0.55 mmol/L) on the PACKS-4 aggregometer (Helena Laboratories, Beaumont, Texas, USA) and the aggregation was assessed photometrically by light transmission aggregometry. Each measurement was repeated 5 times for each sample and the concentration with an intra-assay coefficient of variation in the range of 3.6% to 4.5%. The diagnosis of SPS was confirmed by 2 measurements; the measuring intervals were at least 1 month.

DNA Extraction

Genomic DNA was extracted from peripheral blood with EDTA by SiMax Genomic DNA Extraction kit (SBS Genetech Co, Ltd, Beijing, China) according the manufacturer's instructions. The concentration was estimated by spectrophotometry at 260 nm, adjusted to 30 μg/mL and stored at −20°C for following analysis.

Assessment of the CTGAG Haplotype

The CTGAG haplotype was determined using 5-tagged SNPs (rs1654410, rs1669150, rs1613662, rs12610286, and rs1654431), which were selected by the algorithm

Table 1. Sequencing and HRMA Primers Designed for the Needs of Our Study.

		Annealing Temperature, °C
Sequencing primers		
GP6e1_F	GAGGAAGGGAGGAGAGCATT	62
GP6e1_R	TCCTTTGTCTGGCAGTCCAT	
GP6e2-3_F	AGCAGGCAGGAATGTCCATA	62
GP6e2-3_R	ATGTCCCCCGTATTTGTGTC	
GP6e4_F	GACCTCCCCAGTCTCAGC	66
GP6e4_R	CTTATGGCCCCTCCCTTG	
GP6e5_F	CGGAGATGTTTCGTTATTTGTTC	62
GP6e5_R	GAGAGAGAAGGGGTCCGTGT	
GP6e6_F	TCTCAAAAGGGGAATGGAGA	62
GP6e6_R	AGAGAGCTCCGTCCTCACAC	
GP6e7_F	CGGAGTAGGCACAGTGACAG	62
GP6e7_R	GGAGTTGGCTTTGGTGAAGA	
GP6e8_F	CCCAGATGTGGCTTGGAG	66
GP6e8_R	GTGCCATCCTTCTGTCTTTTC	
HRMA primers		
rs1654416		58
GP6_E237K_F	GAGCATGAAATGCCTGGTTA	
GP6_E237K_R	CGCTGAACTGACCGTCTCAT	
rs2304167		58
GP6_A249T_F	GGGTCTGGAGAGGATGACTT	
GP6_A249T_R	TGTTTGGTTCCCAGAGACTT	
rs1671152		58
GP6_H322N_F	CGGCTGTGAACATCCTGTC	
GP6_H322N_R	CCGCTGACCCGGAAATC	
rs1613662		60
GP6_P219S_F	AGGGGTCCGTGTACCTCA	
GP6_P219S_R	AGGACCCACAGCGACAGA	

Abbreviation: HRMA, high-resolution melting analysis.

implemented in Haploview 4.2 and tested as described by us previously.[15]

Dideoxy Sequencing

The primers used for dideoxy sequencing, designed from GenBank sequence NM_001083899.2 and annealing temperatures are showed in Table 1. Because of the size of the intron 2, only 1 pair of primers for the sequencing of the exons 2 and 3 was designed. The sequencing of the 1634 base pair (bp) fragment embracing the noncoding part of the exon 8 was not done. The polymerase chain reaction (PCR) for sequencing was performed with 100 ng extracted DNA in a final volume of 20 μL using FastStart Taq polymerase (F. Hoffmann-La Roche Ltd, Basel, Switzerland) with 1.5 mM Mg^{2+} concentration. The PCR conditions were 10 minutes for 95°C and 30 cycles at 95°C for 30 seconds, appropriate annealing temperature for 30 seconds and at 72°C for 30 seconds. The PCR products were purified with NucleoSpin Extract II kit (Machery-Nagel GmbH & Co. KG, Düren, Germany) and the sequencing reaction was performed using the BigDye Terminator kit v1.1 (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) at 95°C for 2 minutes and 35 times at 95°C for 15 seconds and at 60°C for 4 minutes. The fragments were run on AB 3500 and subsequently evaluated with DNA Sequencing Analysis Software v5.4 (Applied Biosystems, Foster City, Kalifornia, USA).

High-Resolution Melting Analysis

High-resolution melting analysis was performed in 15 μL reaction with primers designed from GenBank sequence NT_011109.16 as shown in Table 1 using 7.5 μL LighCycler 480 High Resolution Melting Master (F. Hoffmann-La Roche Ltd, Basel, Switzerland), 3 mM final concentration of Mg^{2+} and 0.25 μM of each forward and reverse primer, and 15 ng of template DNA. The annealing temperature is 58°C with the exception of rs1613662 whose annealing temperature was 60°C. There were amplified fragments with the sizes 45 bp (rs1613662), 56 bp (rs1654416), 91 bp (rs2304167), and 59 bp (rs1671152).

Statistical Evaluation

Association analysis was done using SNP and Variation Suite version 8.4 (Golden Helix, Inc, Bozeman, Montana; www.goldenhelix.com); Fisher exact test was selected for allele associations and χ^2 test for haplotype associations. Omega ClustalW2, bioinformatics services on EMBL-EBI Web site[17] was used for the purpose of multiple sequence alignment of sequenced data. The pathogenicity of amino acid changes was determined by 2 computation programs, SIFT and Polyphen2. Variants were considered to be pathogenic if they were predicted as "damaging" by both of these prediction tools.

Table 2. Results of Exon Sequencing of the *GP6* Gene in 18 Patients With SPS and Fetal Loss.[a]

No	H5	rs2288905 c.67+40G>A 55032466	rs892090 c.484A>C 55027704	rs892089 c.495T>C 55027693	rs5030705 c.507G>A 55027681	rs1654425 c.576A>G 55027612	rs1613662 P219S 55025227	rs1654416 E237K 55018667	rs2304167 A249T 55015713
1	Absent	g_g	c_c	c_c	g_g	g_g	S_S	K_K	T_T
2	Present	g_g	c_a	c_t	g_g	g_a	S_P	K_E	T_A
3	Absent	g_a	c_c	c_c	g_g	g_g	S_S	K_K	T_T
4	Absent	g_g	c_c	c_c	g_a	g_g	S_S	K_K	T_T
5	Absent	g_g	c_c	c_c	g_a	g_g	S_S	K_K	T_T
6	Absent	g_g	c_c	c_c	g_a	g_g	S_S	K_K	T_T
7	Present	g_a	c_a	c_t	g_g	g_a	S_P	K_E	T_A
8	Present	g_g	c_a	c_t	g_a	g_a	S_P	K_E	T_A
9	Absent	g_a	c_c	c_c	g_a	g_g	S_S	K_K	T_T
10	Absent	g_g	c_c	c_c	g_a	g_g	S_S	K_K	T_T
11	Absent	g_g	c_c	c_c	a_a	g_g	S_S	K_K	T_T
12	Present	g_g	c_c	c_c	g_g	g_g	S_P	K_E	T_A
13	Absent	g_g	c_c	c_c	g_a	g_g	S_S	K_K	T_T
14	Present	g_g	c_a	c_t	g_a	g_a	S_P	K_E	T_A
15	Present	g_a	c_c	t_t	g_g	g_g	S_P	E_E	A_A
16	Present	g_g	a_a	t_t	g_g	a_a	P_P	E_E	A_A
17	Present	g_a	c_c	c_c	g_g	g_g	S_P	K_E	T_A
18	Present	g_g	c_a	c_t	g_g	g_a	S_P	K_E	T_A

Abbreviations: H5, CTGAG risk haplotype; SPS, sticky platelet syndrome.
[a]Heads of columns contain rs numbers, Human Genome Variation Society (HGVS) names, and chromosomal positions of revealed SNPs.

Results

Patients

Thirty-seven patients with SPS and fetal loss and 42 female healthy control participants had an average age of 37.9 $\pm$ 5.2 and 42.2 $\pm$ 13.0 years, respectively. Eight (21.6%) patients were confirmed as SPS type I, 28 (75.7%) patients had SPS type II, and 1 (2.7%) was diagnosed as SPS type III, according to Mammen criteria.

SNPs in the Coding Region of GP6

The sequencing of the coding region of *GP6* of 18 patients with SPS experiencing fetal loss (9 patients with the risk haplotype CTGAG and 9 patients without its presence) identified heterozygous or homozygous conditions in case of 7 common SNPs within the first 7 coding exons already present in NCBI database of Single Nucleotide Polymorphisms of the National Center for Biotechnology (dbSNP) (build 139). Four of them were synonymous variations, all in exon 4 (rs892090, rs892089, rs5030705, and rs1654425), 3 were nonsynonymous (NS) missense variations in exons 5 (rs1613662), 6 (rs1654416), and 7 (rs2304167), and the SNP rs2288905 was found in intron 2 (Table 2).

Association of the Nonsynonymous Variations With SPS Accompanied by Fetal Loss

To the 3 above-mentioned NS variations, we have included an additional common SNP rs1671152 from exon 8 of the *GP6* gene. All SNPs are tested for the deviation from Hardy-Weinberg equilibrium with no significant findings ($P > .05$),

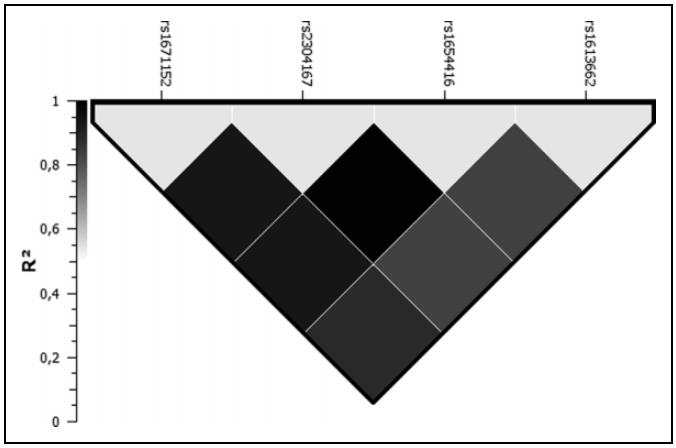

Figure 1. linkage disequilibrium (LD) map of 4 nonsynonymous SNPs of the *GP6* gene.

and all SNPs are in linkage disequilibrium with r^2 ranging from 0.832 to 1 (Figure 1). In total, correlations are rs2304167 and rs1654416. We have tested distribution of alleles between women with fetal loss along with SPS (37), and healthy control women (42) using Fisher exact test and have observed statistically significant results without and with Bonferroni correction. The minor allele ORs range from 3.033 to 5.085 for all 4 SNPs (Table 3). Two SNPs, rs1654416 and rs2304167, have the highest OR about 5.085 (95% confidence interval [CI]: 1.605-16.10) with $P = .003$ and after Bonferroni correction $P = .013$. Therefore, having minor allele and SPS there is about 5 times higher risk to fetal loss event for women. The minor alleles of rs2304167 cause the amino acid substitution of polar threonine to nonpolar alanine (amino acid position

Table 3. Results of Allele Association Analysis of 4 Nonsynonymous SNPs of the *GP6* Gene.

SNP	Minor Allele	Cases (37)	Controls (42)	OR	CI 95%	*P* (Fisher)	*P* (Bonferroni)
		Cases-6 (31)					
rs1613662	P	0.189	0.071	3.033	1.100-8.360	.032	.127
Cases-6		0.161		2.500	0.856-7.297	.110	.438
rs1654416	E	0.203	0.048	5.085	1.605-16.10	.003	**.013**
Cases-6		0.177		4.314	1.303-14.28	.013	.054
rs2304167	A	0.203	0.048	5.085	1.605-16.10	.003	**.013**
Cases-6		0.177		4.314	1.303-14.28	.013	.054
rs1671152	N	0.189	0.048	4.667	1.462-14.89	.006	**.024**
Cases-6		0.161		3.846	1.146-12.91	.025	.102

Abbreviations: CI, confidence interval; OR, odds ratio.
Note. The *p*-values are bold where they are less than or equal to the significance level cut-off of 0.05.

Table 4. Association of Minor Alleles of the NS SNPs With Risk Haplotype *GP6* Gene.[a]

	rs1613662 P[b]		rs1654416 E[b]		rs2304167 A[b]		rs1671152 N[b]	
	H5 Absent	H5 Present	H5 Absent	H5 Present	H5 Absent	H5 Present	H5 Absent	H5 Present
Fetal loss with SPS								
Minor allele absent	24(21)	–	24(21)	–	24(21)	–	24(21)	–
Minor allele present	1(1)	12(9)	1(1)	12(9)	1(1)	12(9)	1(1)	12(9)
Healthy								
Minor allele absent	36	–	37	1	37	1	37	1
Minor allele present	1	5	–	4	–	4	–	4

Abbreviations: H5, CTGAG risk haplotype; NS, nonsynonymous; SPS, sticky platelet syndrome.
[a]Numbers in parentheses denote reduced size of the patients group.
[b]Minor allele mark of specified SNPs.

249) with as ''deleterious—low confidence'' considered SIFT score of 0.01; however, the PolyPhen2 score comprises 0.093, which is the highest of all NS variations and considered as ''benign.'' However, rs1671152 minor allele shows OR: 4.667 (95% CI: 1.462-14.89) with the P value of .006 (after Bonferroni correction: 0.024). The lowest association has been shown by rs1613662 (OR: 3.033, 95% CI: 1.100-8.360, and $P = .032$; after Bonferroni correction, $P = .127$).

Association Between NS Variations and Risk Haplotype

The NS variations are associated with the risk haplotypes as shown in Table 4. Almost the whole group without risk haplotype (24 from 25) and none from the sequenced group with risk haplotype have a major homozygous genotype for all 4 NS variations. All patients harboring the risk haplotypes (12 cases) are carriers of minor allele for all 4 NS SNPs. This finding was confirmed by the testing of 42 healthy control participants with known-risk haplotypes for genotypes on the loci of NS variants. For rs1654416, rs2304167, and rs1671152, all 37 women without risk haplotype and 1 from 5 risk haplotype carriers harbor a major homozygous genotype for all 3 NS variations. For rs1613662, all 5 women with risk haplotype are heterozygotes. One healthy women without risk haplotype is heterozygote for rs1613662 too. Using the EM algorithm, the estimated minor haplotype with predicted protein residues PEAN (rs1613662, rs1654416, rs2304167, rs1671152) was significantly associated with the given phenotype of SPS accompanied by fetal loss (OR: 4.74, 95% CI: 1.486-15.15; Table 5). After exclusion of patients with 1 abortion, the PEAN haplotype is still significantly associated with the fetal loss (OR: 3.922, 95%CI: 1.168-13.17; Table 5).

Table 5. Results of NS Haplotype Association Analysis.[a]

NS Haplotype	Cases (37)	Controls (42)	*P*	χ^2	OR	95% CI
	Cases-6 (31)					
SKTH	0.797	0.929	.024	5.089	0.324	0.117-0.893
Cases-6	0.823		.079	3.080	0.392	0.134-1.146
PEAN	0.189	0.048	.005	7.997	**4.746**	**1.486-15.15**
Cases-6	0.161		.019	5.481	**3.922**	**1.168-13.17**
PKTH	0.000	0.024	.185	1.761	–	–
Cases-6	0.000		.225	1.473	–	–

Abbreviations: CI, confidence interval; NS, nonsynonymous; OR, odds ratio.
[a]Order of NS SNPs in haplotypes: rs1613662, rs1654416, rs2304167, rs1671152.
Note. The bold values represent significant associations at *p*-value cut-off level less than or equal to 0.05.

Discussion

The GPVI is a platelet membrane GP, which is receptor for collagen and convulxin, and is important in collagen-induced platelet aggregation. Increased attention was devoted to the

genetic association analysis of GPVI with SPS. Some reports have identified the prevalence of selected *GP6* SNPs as independent risk factors for deep vein thrombosis (DVT) in patient with platelet hyperaggregability.[14] Sokol et al[15] has found that some haplotypes of *GP6* such as CTGAG represent a risk factor in patients with SPS and fetal loss. Our report presents a pilot study regarding the analysis of NS changes in the coding region of the *GP6* gene in association with SPS and fetal loss. We have shown that haplotype PEAN is associated with this condition (OR: 4.746 (95% CI: 1.486-15.15, $P = .005$).This finding extends the previously described association of SPS and fetal loss with 2 *GP6* risk haplotypes CTGAG and CGATAG.[15]

Different mechanisms can be considered for the impairment of platelet function. Within the coding region of *GP6*, some pathogenic mutations leading to the platelet-type bleeding disorder type 11 (BDPLT11) are reported, resulting in defective binding to collagen.[18,19] It was reported that these mutations resulted in decreased collagen-induced activation and aggregation of platelets in vitro mostly presented as incomplete deficiency of GPVI. Although an understanding of the rare disorders of platelet aggregation has emerged, the role of common genetic variations contributing to platelet aggregation is less known. However, some SNPs were found to be associated with thrombophilic conditions.[14,20] In this study, we identified 4 NS variants within the coding region of the *GP6* gene, which were significantly associated with the SPS phenotype accompanied by fetal loss. The authors and others showed independently that these variants are in linkage disequilibrium and create minor haplotypes at the protein level with protein residues PEAN (in successive order—rs1613662, rs1654416, rs2304167, and rs1671152), which was determined in this study, and PEALN (rs1613662, rs1654416, rs2304167, rs1654413, and rs1671152) as determined in a previous study.[21] Using tools for annotation of coding NS SNPs, we found out that rs2304167 minor allele causes the amino acid substitution of polar threonine to nonpolar alanine (p. T249A) with as damaging considered SIFT score 0.01; however, the PolyPhen2 score comprises 0.093 assumed as benign. Three of these variants (rs1613662, rs1654416, and rs2304167) are localized in the extracellular topological protein domain, which is not involved in the interaction of GPVI with collagens or convulxin.[22] In a functional study, PEALN haplotype revealed a significant difference in membrane expression of GPVI;[21] however, a subsequent study has shown that this accounts for no more than 16% of the difference in expression level of GPVI.[23] Transfection experiments were performed to evaluate molecular basis for qualitative differences in the function of these GPVI isoforms. The cytoplasmic variation in the codon 322 (rs1671152) causing missense substitution N322H was used in transfection experiments with the GPVI isoform carrying the haplotype PEALN. It was demonstrated that although L317 increases binding to calmodulin, N322 attenuates binding to Fyn/Lyn, resulting in the significant decrease in the tyrosine phosphorylation of Syk.[24] This findings argue that the PEALN haplotype has a significant effect on GPVI-mediated signal transduction, which could be true for the PEAN haplotype as well. Particularly, the L317 can be responsible for increased binding to calmodulin, which is suggested to be responsible for decreased production of soluble GPVI.[24] Because soluble GPVI has been shown in some cases to be an efficient inhibitor of thrombus formation in vitro and in vivo,[25] this could be one of the explanations for the association of this allele with increased risk for thrombosis.[26] On the contrary, the interaction of Fyn and Lyn with the proline-rich region of the GPVI is critical for maximal signaling by GPVI,[27] and N322 results in decreased Syk phosphorylation. It is known that Syk inhibition can improve responses to vascular injury without affecting hemostasis. Specifically, lack of Syk attenuated shear-induced thrombus formation, which makes it an appropriate target for the therapy.[28] Hypercoagulable state is associated with physiological pregnancy; however, genetic factors are considered to increase the severity of this condition. Therefore, the role of the decreased GPVI signaling in thrombosis caused by genotype and haplotype differences needs to be assessed in the subsequent studies on larger cohorts. Moreover, functional studies of ligands and pathways of GPVI, which are going to analyze different isoforms, have to be performed. Additionally, as mentioned above, some *GP6* SNPs were associated with DVT in patients with SPS. Therefore, it needs to be evaluated if the general population of patients with DVT shows the association with the PEAN haplotype, or if this association is linked to the limited patient population with SPS.

Declaration of Conflicting Interests

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Supplemental Material

Supplementary material for this article is available online.

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