

# Correlation between factor VII and PAI-1 genetic variants and recurrent miscarriage

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## ABSTRACT

**Background:** Polymorphisms which are presented below may be the cause of inherited thrombophilia and may result in pregnancy loss. The hypothesis is based on a number of cardiology studies which have confirmed the involvement of these polymorphisms in thrombotic incidents.

**Objectives:** To evaluate the role of polymorphisms of factor VII gene (*Arg353Gln*, -122T > C) and PAI-1 gene (-675 4G/5G) in the etiology of recurrent miscarriage.

**Material and methods:** The study group included 152 women with a positive history of  $\geq 2$  consecutive pregnancy losses (114 and 38 women with 2 and  $\geq 3$  miscarriages, respectively), while 180 healthy women were recruited as controls. Genetic analysis was performed with the use of PCR/RFLP.

**Results:** Lower frequency of *Arg353/Gln353* was observed in women with 2 and  $\geq 3$  miscarriages as compared to controls (21.1% vs. 23.9% and 13.2% vs. 23.9%, respectively). The frequency of *Gln353* was lower in women with  $\geq 3$  miscarriages as compared to controls (6.6% vs. 11.9%,  $p = ns$ ). The frequency of -122TT was higher in women with  $\geq 3$  miscarriages as compared to controls (86.84% vs. 76.67%,  $p = ns$ ), whereas -122TC was more frequent in controls (13.16% vs. 22.78% in controls,  $p = ns$ ). The frequency of -122T was higher in patients with  $\geq 3$  abortions as compared to controls (93.42% vs. 88.06%,  $p = ns$ ), and -122C was observed more frequently in controls (6.58% vs. 11.94% in controls,  $p = ns$ ). There were no significant differences as far as the -675 4G/5G polymorphism was concerned.

**Conclusions:** The obtained results suggest a possible protective role of *Gln353* and -122C alleles in recurrent miscarriage.

**Key words:** thrombophilia, pregnancy loss, gene polymorphism

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## INTRODUCTION

The etiology of recurrent miscarriage may be identified in only about 30–50% of the affected patients. Importantly, numerous studies have already proven the existence of a correlation between inherited thrombophilia and pregnancy loss [1–6]. Standard diagnostic testing for thrombophilia in these patients involves factor V Leiden, 677C>T *MTHFR* gene polymorphism, 20210G>A prothrombin gene polymorphism, protein C, protein S and antithrombin de-

ficiency. However, anticoagulant treatment with heparin and acetylsalicylic acid is successful in the absence of the abovementioned genetic impairments and after exclusion of the antiphospholipid syndrome, suggesting the existence of other genetic variants, which are not included in the basic diagnostic algorithm, conditioning inherited thrombophilia.

Factor VII, together with the tissue factor, takes part in the activation of the exogenous path of clotting and formation of a blood clot after vessel injury. Gene encoding factor

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VII is located on the short arm of chromosome 13 and contains 9 exons (NM\_000131). The majority of the identified factor VII gene polymorphisms are correlated with factor VII deficiency, resulting in exaggerated bleeding and constituting a risk factor for massive hemorrhage. However, several polymorphisms increase factor VII activity and its higher concentration in serum has been described. It may result in an increased risk for thrombotic changes and, in consequence, recurrent miscarriage [7, 8].

Plasminogen activator inhibitor-1 (PAI-1) is the main physiological inhibitor of tissue plasminogen activator and urokinase plasminogen activator. It plays a vital role in the regulation of fibrinolytic processes and increased PAI-1 concentration may be the cause of thrombophilia. The gene encoding PAI-1 (*SERPINE1*) is located on the short arm of chromosome 7 and contains 9 exons (NM\_000602). Genetically conditioned changes in PAI-1 serum concentration and activity may result in hypofibrinolysis, thrombotic changes in the utero-placental unit, and elevated risk for recurrent pregnancy loss. Enhanced PAI-1 secretion by endothelial cells leads to the formation of thrombus in the spiral arteries. Apart from the correlation with recurrent miscarriage, PAI-1 gene polymorphisms may be connected with the development of preeclampsia, gestational hypertension, intrauterine growth restriction, or intrauterine fetal death [9, 10].

The aim of our research was to investigate the possibility of a correlation between selected genetic variants of factor VII (*Arg353Gln* and *-122T>C*) and PAI-1 (*-675 4G/5G*) and recurrent miscarriage. Polymorphisms which have been presented below may be the cause of inherited thrombophilia and may result in pregnancy loss. The hypothesis is based on a number of cardiology studies which have confirmed the involvement of these polymorphisms in thrombotic incidents [11–18].

## MATERIAL AND METHODS

All participants (Polish origin, Caucasian race) were recruited at the Department of Perinatology and Wom-

en's Diseases, Poznan University of Medical Sciences, between 2011–2013. A total of 152 women (mean age  $30.16 \pm 3.82$  years, range 21–45 years, median 30 years) with a history of  $\geq 2$  consecutive miscarriages were enrolled into the study. Spontaneous abortion was defined as the loss of pregnancy before 22 completed weeks of gestation. Patients with known causes of recurrent miscarriage (anatomical anomalies of the genitourinary tract, chromosomal defect, chronic disease, infection, hormonal impairment, antiphospholipid antibodies, thromboembolic disease, cervical insufficiency or other obstetric complications which could cause miscarriage, e.g. hypertension, preeclampsia, eclampsia, gestational diabetes mellitus) were excluded. The study group was subdivided based on the number of spontaneous abortions: 114 with 2 and 38 women with  $\geq 3$  consecutive miscarriages.

The control group included 180 women (mean age  $29.46 \pm 4.26$  years, range 19–42 years, median 29 years) with a positive history of  $\geq 1$  uncomplicated pregnancy (healthy term newborn). All women with a history of miscarriage, other obstetric complications which might be related to thrombotic changes (preeclampsia, fetal hypotrophy, preterm delivery, preterm placental ablation, intrauterine fetal death), thromboembolic disease, and chronic diseases were excluded.

Genotyping of *Arg353Gln* and *-122T>C* factor VII gene polymorphisms and *-675 4G/5G PAI-1* gene polymorphism was performed for the entire study population. Genetic analysis was performed with the use of polymerase chain reaction/restriction fragments length polymorphism method (PCR/RFLP). Genomic DNA was extracted from blood leucocytes using QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany). Starters used for amplification and restriction enzymes used to obtain better resolution for visualization are shown in Table 1. The analysis of the digested fragments was performed using 2% agarose gel electrophoresis.

Statistical analysis was performed using SPSS 22.0 PL for Windows. Frequencies of genotypes were compared with chi-square test (one-sided Fisher test). The expected

**Table 1** Starters and restriction enzymes used in genetic analysis

	Starters	Restriction enzyme	Recognized sequence	Digested fragments
<i>Arg353Gln</i>	F 5'-GGG AGA CTC CCC AAA TAT CAC-3' R 5'-ACG CAG CCT TGG CTT TCT CTC-3'	<i>MspI</i> (EURx)	C <sup>^</sup> CGG	<i>Arg353/Arg353</i> – 206, 67, 39 bp <i>Arg353/Gln353</i> – 273, 206, 67, 39 bp <i>Gln353/Gln353</i> – 273, 39 bp
<i>-122T&gt;C</i>	F 5'-TCG CAT GAT TGC TAT GGG AC-3' R 5'-GTT GAC ATT CCC CAT GGG AC-3'	<i>SclI</i> ( <i>Bsp1286I</i> ) (Thermo Scientific)	GDGCH <sup>^</sup> C	<i>-122TT</i> – 200 (210), 156 bp <i>-122TC</i> – 200 (210), 156, 90, 66 bp <i>-122CC</i> – 200 (210), 90, 66 bp
<i>-675 4G/5G</i>	5'-GCC CTC AGG GGC ACA GAG AGA GTC TGG CCA-3' 5'-GCA ATG CAG CCA GCC ACG TG — 3'	<i>BsII</i> (Thermo Scientific)	CCNNNNN <sup>^</sup> NNGG	<i>-675 4G</i> 107, 56 bp <i>-675 5G</i> 74, 56, 34 bp

genotype frequencies were calculated from allele frequencies with the Hardy-Weinberg equation.

All patients gave their written confirmed consent for the study. Local Ethics Committee approved of the study (no. 422/11).

## RESULTS

### *Arg353Gln* factor VII gene polymorphism

The frequency of the *Arg353/Arg353* genotype was slightly higher in women with 2 miscarriages (78.0% vs. 76.1% in controls,  $p = ns$ ). The *Arg353/Gln353* genotype was observed more frequently in controls as compared to women with 2 pregnancy losses (21.1% vs. 23.9% in controls,  $p = ns$ ). The mutated *Gln353/Gln353* genotype was present only in 1 patient from that subgroup and in none of the controls (0.9% vs. 0.0% in controls,  $p = ns$ ). There were no statistically significant differences in the frequency of alleles between both analyzed groups (*Arg353*: 88.6% vs. 88.1% in controls,  $p = ns$ ; *Gln353*: 11.4% vs. 11.9% in controls,  $p = ns$ ) (Table 2).

The differences between genotype and allele frequencies were more clear between the group with  $\geq 3$  miscarriages and controls. The homozygotic *Arg353/Arg353* genotype was slightly more frequent in the group with  $\geq 3$  miscarriages (86.8% vs. 76.1% in controls,  $p = ns$ ). The study revealed an overrepresentation of heterozygotic *Arg353/Gln353* genotype in controls (23.9% in controls vs. 13.2%,  $p = ns$ ). The frequency of the *Arg353* allele was slightly higher in the group with  $\geq 3$  spontaneous abortions (93.4% vs. 88.1% in controls,  $p = ns$ ) and the mutated *Gln353* allele was more frequent in controls (11.9% in controls vs. 6.6%,  $p = ns$ ) (Table 2).

### *-122T>C* factor VII gene polymorphism

The frequency of genotypes and alleles of the *-122T>C* polymorphism was similar in the group with 2 miscarriages and in controls (*-122TT*: 78.07% vs. 76.67% in controls,  $p = ns$ ; *-122TC*: 20.18% vs. 22.78% in controls,  $p = ns$ ; *-122CC*: 1.75% vs. 0.55% in controls,  $p = ns$ ; *-122T*: 88.16% vs. 88.06% in controls,  $p = ns$ ; *-122C*: 11.84% vs. 11.94% in controls,  $p = ns$ ) (Table 3).

Slightly higher frequency of the homozygotic *-122TT* genotype was observed in the group with  $\geq 3$  pregnancy losses (86.84% vs. 76.67% in controls,  $p = ns$ ), and the heterozygotic *-122TC* genotype was found more frequently in controls than in women with  $\geq 3$  miscarriages (13.16% vs. 22.78% in controls,  $p = ns$ ). *-122T* allele was more frequent in the study group of women with 3 or more miscarriages (93.42% vs. 88.06% in controls,  $p = ns$ ) and the mutated *-122C* allele — in controls (6.58% vs. 11.94% in controls,  $p = ns$ ) (Table 3).

### *-675 4G/5G PAI-1* gene polymorphism

The frequency of genotypes and alleles was comparable in the group with 2 miscarriages and in controls (*-675 4G/-675 4G*: 32.5% vs. 35.0%,  $p = ns$ ; *-675 4G/-675 5G*: 49.1% vs. 47.%,  $p = ns$ ; *-675 5G/-675 5G*: 18.4% vs. 17.8%,  $p = ns$ ; allele *-675 4G*: 57.0% vs. 58.6%,  $p = ns$ ; *-675 5G*: 43.0% vs. 41.4%,  $p = ns$ ) (Table 4). Similar observation was made for the group with  $\geq 3$  pregnancy losses (*-675 4G/-675 4G*: 34.2% vs. 35.0%,  $p = ns$ ; *-675 4G/-675 5G*: 50.0% vs. 47.2%,  $p = ns$ ; *-675 5G/-675 5G*: 15.8% vs. 17.8%,  $p = ns$ ; allele *-675 4G*: 59.2% vs. 58.6%,  $p = ns$ ; *-675 5G*: 40.8% vs. 41.4%,  $p = ns$ ) (Table 4).

**Table 2.** The frequency of genotypes and alleles of the *Arg353Gln* factor VII gene polymorphism in the groups with a history of 2 and  $\geq 3$  miscarriages

<i>Arg353Gln</i>	Study group (n = 152)								Control group (n = 180)	
	2 miscarriages (n = 114)				$\geq 3$ miscarriages (n = 38)				Observed value n [%]	Expected value [%]
Genotypes	Observed value n [%]	Expected value [%]	OR	P	Observed value n [%]	Expected value [%]	OR	P		
<i>Arg353/Arg353</i>	89 (78.0)	78.5	1.12	0.40	33 (86.8)	87.2	2.07	0.10	137 (76.1)	77.5
<i>Arg353/Gln353</i>	24 (21.1)	20.2	0.85	0.34	5 (13.2)	12.3	0.48	0.10	43 (23.9)	21.1
<i>Gln353/Gln353</i>	1 (0.9)	1.3	–	0.39	0 (0.0)	0.5	–	1	0 (0.0)	1.4
Total	114 (100.0)	100			38 (100.0)	100.0			180 (100.0)	100
Alleles										
<i>Arg353</i>	202 (88.6)	–	1.05	0.48	71 (93.4)	–	1.93	0.12	317 (88.1)	–
<i>Gln353</i>	26 (11.4)	–	0.95	0.48	5 (6.6)	–	0.52	0.12	43 (11.9)	–
Total	228 (100.0)	–			76 (100.0)	–			360 (100.0)	–

Analyzed subgroups were compared to the control group

**Table 3.** The frequency of genotypes and alleles of the -122T>C factor VII gene polymorphism in the groups with a history of 2 and ≥ 3 miscarriages

-122T>C	Study group (n = 152)								Control group (n = 180)	
	2 miscarriages (n = 114)				≥ 3 miscarriages (n = 38)				Observed value n [%]	Expected value [%]
Genotypes	Observed value n [%]	Expected value [%]	OR	P	Observed value n [%]	Expected value [%]	OR	P		
TT	89 (78.07)	77.72	1.08	0.45	33 (86.84)	87.27	2.01	0.12	138 (76.67)	77.54
TC	23 (20.18)	20.88	0.86	0.35	5 (13.16)	12.29	0.51	0.13	41 (22.78)	21.03
CC	2 (1.75)	1.40	3.20	0.33	0 (0.00)	0.43		0.83	1 (0.55)	1.43
Total	114 (100.0)	100.0			38 (100.0)	100.0			180 (100.00)	100
Alleles										
T	201 (88.16)	–	1.01	0.54	71 (93.42)	–	1.93	0.12	317 (88.06)	–
C	27 (11.84)	–	0.99	0.54	5 (6.58)	–	0.52	0.12	43 (11.94)	–
Total	228 (100.0)	–			76 (100.0)	–			360 (100.0)	–

Analyzed subgroups were compared to the control group

**Table 4.** The frequency of genotypes and alleles of the -6754G/-675 5G PAI-1 gene polymorphism in the groups with a history of 2 and ≥ 3 miscarriages

-675 4G/5G	Study group (n = 152)								Control group (n = 180)	
	2 miscarriages (n = 114)				≥ 3 miscarriages (n = 38)				Observed value n [%]	Expected value [%]
Genotypes	Observed value n [%]	Expected value [%]	OR	P	Observed value n [%]	Expected value [%]	OR	P		
4G/4G	37 (32.5)	32.5	0.89	0.37	13 (34.2)	35.0	0.97	0.54	63 (35.0)	34.4
4G/5G	56 (49.1)	49.0	1.08	0.42	19 (50.0)	48.3	1.12	0.45	85 (47.2)	48.5
5G/5G	21 (18.4)	18.5	1.04	0.50	6 (15.8)	16.7	0.87	0.49	32 (17.8)	17.1
Total	114 (100.00)	114 (100.0)			38 (100.0)	38 (100.0)			180 (100.0)	100
Alleles										
4G	130 (57.0)	–	0.94	0.38	45 (59.2)	–	1.03	0.51	211 (58.6)	–
5G	98 (43.0)	–	1.07	0.38	31 (40.8)	–	0.98	0.51	149 (41.4)	–
Total	228 (100.0)	–			76 (100.0)	–			360 (100.0)	–

Analyzed subgroups were compared to the control group

## DISCUSSION

### Arg353Gln factor VII gene polymorphism

To the best of our knowledge, there has been only one report on the correlation of the Arg353Gln factor VII gene polymorphism and the risk of recurrent miscarriage, i.e. the study by Seremak-Mrozikiewicz et al. [7], so far. Our study has been the second analysis on the correlation between the Arg353Gln factor VII gene polymorphism and recurrent pregnancy loss. Seremak-Mrozikiewicz et al., based their research hypothesis on a number of cardiology reports, suggesting the existence of a correlation between the abovementioned

genetic variant and elevated risk for cardiovascular disease. It is believed that the presence of the mutated Gln353 allele leads to decreased concentration and activity of factor VII, resulting in lower intensity of coagulation cascade and reduced risk for thrombotic changes [7, 19].

In the cited paper (by Seremak-Mrozikiewicz), the study group included 104 patients with ≥ 2 miscarriages between 6–13 weeks of gestation and 163 healthy controls with a negative history of pregnancy loss and a positive history of ≥ 1 uncomplicated pregnancy (healthy newborn at term). Their study revealed a slightly higher frequency of the Arg353/Arg353 genotype in the study group (89.69%

vs. 74.5% in controls,  $p = 0.086$ ). Moreover, overrepresentation of the *Arg353/Gln353* genotype was observed in controls (17.31% vs. 25.15% in control group,  $p = 0.086$ ). The frequency of the mutated *Gln353* allele was also higher in controls (12.58% in controls vs. 8.65%,  $p = 0.101$ ). Further analysis revealed a lower frequency of the *Arg353/Gln353* genotype in the group with 2 miscarriages, as well as  $w \geq 3$  miscarriages as compared to controls (respectively, 16.25% and 20.83% vs. 25.15%) [7].

The obtained results suggest a possible protective role of the mutated *Gln353* allele of the *Arg353Gln* factor VII gene polymorphism in recurrent miscarriage. Similar conclusions were reported by Seremak-Mrozikiewicz et al.

#### **-122T>C factor VII gene polymorphism**

To the best of our knowledge, the literature offers no reports on the correlation between -122T>C factor VII gene polymorphism and recurrent miscarriage and our study has been the first on the topic in question. It points out a possible protective role of the mutated -122C allele of the -122T>C factor VII gene polymorphism in the occurrence of recurrent abortions, especially in women with  $\geq 3$  pregnancy losses. It is indicated by a slightly higher frequency of the -122TT genotype in the group with  $\geq 3$  miscarriages (86.84% vs. 76.67% in controls,  $p = 0.12$ ) and overrepresentation of the -122TC genotype in controls (13.16% vs. 22.78% in controls,  $p = 0.13$ ). Moreover, the frequency of the -122T allele was higher in the group with  $\geq 3$  pregnancy losses (93.42% vs. 88.6% in controls,  $p = 0.12$ ) and the mutated -122C allele was noted more frequently in controls (6.58% vs. 11.94% in controls,  $p = 0.12$ ).

#### **-675 4G/5G PAI-1 gene polymorphism**

The abovementioned polymorphism is one of the most often studied PAI-1 functional genetic variants as far as its correlation with obstetric complications is concerned. The -675 4G allele determines increased PAI-1 concentration and enhanced prothrombotic activity. The -675 4G/5G polymorphism has been suggested to be involved in the etiology of intrauterine fetal death, intrauterine growth restriction, preterm placental abruption, recurrent miscarriage, and preeclampsia [20, 21].

Increased PAI-1 concentration and reduction of fibrinolysis is observed in carriers of the homozygotic -675 4G/-675 4G genotype as compared to carriers of the -675 5G/-675 5G genotype. Intermediate value of the PAI-1 concentration is noted in carriers of the heterozygotic -675 4G/-675 5G genotype. So, it is believed that the mutated -675 5G allele may play a protective role in recurrent pregnancy loss. In our study, no significant differences between the analyzed groups were observed. The conducted analysis did not re-

veal any correlation of the -675 4G/5G PAI-1 gene polymorphisms with the risk of recurrent miscarriage. However, numerous contemporary studies have confirmed the influence of the abovementioned genetic variant on elevated risk for recurrent pregnancy loss.

The aim of the research by Subrt et al., was to evaluate the frequency of genotypes and alleles of the -675 4G/-675 5G PAI-1 gene polymorphism and its correlation with the presence of antiphospholipid antibodies in a group of 157 women with a positive history of recurrent spontaneous abortion. The study revealed a statistically significant connection between this obstetric complication and the -675 4G/-675 4G genotype. Regardless, no correlation with the presence of antiphospholipid antibodies was found [22].

The study by Ivanov et al., also suggests an independent role of the -675 4G/-675 5G PAI-1 gene polymorphism in the etiology of recurrent pregnancy loss. Their study population included 110 women with a history of recurrent miscarriage and 97 healthy controls. These authors evaluated the frequency of the -675 4G/-675 5G PAI-1 gene polymorphism, 20210G>A prothrombin gene polymorphism, 677C>T MTHFR gene polymorphism and factor V Leiden. Higher frequency of the -675 4G/-675 5G PAI-1 gene polymorphism was noted in the study group (41.8% vs. 26.8%, WR 1.96;  $p = 0.034$ ). Moreover, this difference remained significant even after the exclusion of the carriers of the 20210G>A prothrombin gene polymorphism, 677C>T MTHFR gene polymorphism and factor V Leiden from the analysis [23].

Coulam et al., conducted a very interesting analysis on genetically conditioned thrombophilia and its correlation with recurrent pregnancy loss. These authors based their hypothesis on the well-documented fact that inherited thrombophilia constitutes a risk factor for cardio-vascular diseases and obstetric complications. The aim of their study was to compare the frequency of nine mutations correlated with thrombophilia in a group of 550 women with a history of recurrent miscarriage, in 43 patients with deep vein thrombosis, and in 41 healthy controls. Each patient was genotyped for the presence of the 1691G>A and H1299R factor V gene polymorphism, 20210G>A prothrombin gene polymorphism, V34L factor XIII gene polymorphism, -455G>A beta-fibrinogen gene polymorphism, 677C>T and 1298A>C MTHFR gene polymorphism, and -675 4G/5G PAI-1 gene polymorphism. The frequency of all genetic variants was higher in patients with deep vein thrombosis as compared to controls and women with recurrent miscarriage. The V34L factor XIII gene polymorphism and -675 4G/5G PAI-1 gene polymorphism were statistically more often observed in the group with pregnancy loss as compared to controls. These authors concluded that basic testing for thrombophilia should also include the -675 4G/5G PAI-1 gene polymorphism [24].



## CONCLUSIONS

1. The obtained results suggest a possible protective role of the mutated *Gln353* allele of the *Arg353Gln* factor VII gene polymorphism in recurrent miscarriage.
2. The present study points out a possible protective role of the mutated *-122C* allele of the *-122T>C* factor VII gene polymorphism as far as the occurrence of recurrent abortion is concerned, especially in the group with  $\geq 3$  pregnancy losses.
3. The analysis did not reveal any correlations between *-675 4G/5G PAI-1* gene polymorphisms and the risk for recurrent miscarriage.
4. A correlation between thrombophilia and recurrent miscarriage has been observed by numerous authors. However, the pathological mechanisms involved in the process remain poorly understood. The etiology of recurrent pregnancy loss is multifactorial and efficient treatment remains a challenge. Further studies are essential in order to obtain strong evidence.

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