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The -*323P0/P10* factor VII gene polymorphism and the risk of recurrent miscarriage

Polimorfizm -*323P0/P10* genu czynnika VII krzepnięcia a ryzyko poronień nawracających

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Abstract

Objectives: *Genetically determined disturbances in the activity of coagulation factor VII may lead to obstetric complications. The aim of the study was to evaluate the correlation between -323P0/P10 factor VII gene polymorphism and the risk of recurrent miscarriage.*

Material and methods: *The study group consisted of 152 women with a history of ≥2 miscarriages. The control group comprised 180 women with no history of miscarriage and ≥1 pregnancy who gave birth to a healthy newborn at term. The study group was further subdivided twice into two subgroups: 114 patients with a history of 2 miscarriages and 38 subjects with a history of ≥3 miscarriages, and 123 patients with miscarriages <13 gw. and 29 with miscarriages <21 gw. Genetic analysis was performed with the use of PCR/RFLP.*

Results: *Overrepresentation of P0/P0 genotype and lower frequency of P0/P10 genotype was noted in the study group as compared to controls (P0/P0: 80,26 vs. 76,67%, p=0,25; P0/P10: 18,42 vs. 22,78%, p=0,20). A higher presentation of P0/P0 genotype and P0 allele, lower frequency of P0/P10 genotype and P10 allele was observed in the subgroup of women with ≥3 miscarriages as compared to controls (P0/P0: 86,84 vs. 76,67%, p=0,12; P0: 93,42 vs. 88,06%, p=0,12; P0/P10:13,16 vs. 22,78%, p=0,13; P10: 11,94 vs. 6,58%, p=0,12).*

Conclusions: *The obtained results suggest a probable protective role of -323P10 allele against the risk of miscarriage in women with ≥3 recurrent pregnancy losses.*

Key words: **recurrent miscarriage** / **coagulation factor VII** / **genetic polymorphism** /

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Streszczenie

Cel pracy: *Genetycznie uwarunkowane zaburzenia aktywności VII czynnika krzepnięcia mogą prowadzić do powikłań położniczych. Celem pracy była ocena związku polimorfizmu -323P0/P10 genu czynnika VII krzepnięcia z występowaniem poronień nawracających.*

Materiał i metody: *Do grupy badanej włączono 152 kobiety z wywiadem obciążonym wystąpieniem ⩾2 poronień. Grupę kontrolną stanowiło 180 kobiet (⩾1 ciąża zakończona urodzeniem zdrowego, donoszonego noworodka, brak poronień w wywiadzie). Grupę badaną dwukrotnie podzielono na dwie podgrupy (114 pacjentek z 2 poronieniami oraz 38 z ⩾3 poronieniami, 123 pacjentki z poronieniami <13 tc. oraz 29 z poronieniami <21 tc.). Analizę genetyczną przeprowadzono przy użyciu metody PCR/RFLP.*

Wyniki: *Odnotowano wyższą częstość genotypu P0/P0 oraz niższą P0/P10 w grupie badanej w porównaniu do kontroli (P0/P0: 80,26 vs. 76,67%, p=0,25; P0/P10: 18,42 vs. 22,78%, p=0,20). W podgrupie z ⩾3 poronieniami zaobserwowano znacznie wyższą frekwencję genotypu P0/P0 i allela P0 oraz niższą częstość genotypu P0/P10 i allela P10 w porównaniu do kontroli (P0/P0: 86,84 vs. 76,67%, p=0,12; P0: 93,42 vs. 88,06%, p=0,12; P0/P10:13,16 vs. 22,78%, p=0,13; P10: 11,94 vs. 6,58%, p=0,12).*

Wnioski: *Analiza częstości występowania genotypów i alleli polimorfizmu insercyjno-delecyjnego -323P0/P10 genu czynnika VII krzepnięcia pozwala na założenie, że zmutowany insercyjny allel -323P10 odgrywa protekcyjną rolę w stosunku do występowania poronień u kobiet z 3 i więcej utratami ciąży.*

Słowa kluczowe: **poronienia nawracające** / **czynnik VII krzepnięcia** / / **polimorfizm genetyczny** /

Introduction

Factor V Leiden, increased prothrombin concentration and methylenetetrahydrofolate reductase defects are well-known reasons of inherited thrombophilia. All of them are involved in the augmentation of endovascular clotting and lead to pregnancy loss or failed pregnancy course [1, 2]. Current studies focus on a possible correlation of genetic variants of coagulation factor VII and an increased risk of recurrent pregnancy loss [3, 4].

Factor VII occupies the central position in the coagulation cascade and together with the tissue factor play an important role in the activation of exogenous path of clotting and formation of a blood clot after vessel injury. The polymorphous gene encoding factor VII is located on the short arm of chromosome 13 and contains 9 exons. The majority of genetic variants is connected with factor VII deficiency, which is the cause of exaggerated bleedings in patients. However, a few polymorphisms increase factor VII activity and its higher concentration in serum has been described. These polymorphisms could also enhance the risk of thrombotic changes and, in consequence, recurrent miscarriages. The greatest significance in this respect is being assigned to the functional polymorphism causing the exchange of arginine to glutamine in position 353 of the factor VII protein chain (*Arg353Gln*) [5, 6].

The *-323P0/P10* polymorphism is one of the variants influencing the regulation of factor VII concentration and activity. It refers to the insertion of 10 bp (CCTATATCCT) in the promoter region. The *-323P0/P10* polymorphism was shown to be functionally relevant and the presence of *-323P10* insertion allele is correlated with decreased factor VII activity. This rare genetic variant probably decreases promoter activity. The most interesting fact is that the *-323P0/P10* polymorphism remains in linkage disequilibrium with other variants which affect factor VII serum concentration [7, 8]. In this way, the presence of *-323P0/P10* polymorphism most likely modulates the activity and concentration of factor VII. Linkage disequilibrium between the *-323P0/P10* and *Arg353Gln* polymorphisms has been suggested [9].

Establishing a connection between factor VII and obstetric complications has been the aim of our research. The majority of studies recruit women with genetically conditioned factor VII deficiency and an increased risk of dangerous hemorrhage during pregnancy, delivery, and puerperium. Only few studies focus on the changes in factor VII activity as a cause of recurrent miscarriage.

Aim of the study

The aim of the study was to evaluate the correlation between *-323P0/P10* factor VII gene polymorphism and the risk of recurrent miscarriage.

Material and methods

The patients were enrolled in the study between 2011-2013, at the Division of Perinatology and Women's Diseases, University of Medical Sciences, Poznan, Poland.

All subjects gave their written informed consent to participate in the project. The goals of the investigation were approved by the Bioethics Committee of Poznan University of Medical Sciences (nr 422/11). All women were Caucasian and of Polish origin.

Study group

The study group consisted of 152 women with a history of two or more miscarriages (mean age 30,16±3,82 years, range 21-45 years, median 30 years). Miscarriage was defined as the loss of pregnancy before the end of 22 gestational weeks (gw). Gestational age at the time of abortion was established on the basis of the date of the last menstruation, regularity of periods and ultrasound examinations.

All patients with known reasons for miscarriage (anatomical anomalies of genito-urinary tracts, chromosomal defects, chronic diseases, infections, hormonal impairments), cervical insufficiency or other obstetric complications that could be a reason for miscarriage (gestational hypertension, eclampsia, preeclampsia, gestational diabetes) were excluded from the analysis.

The study group was divided twice into two subgroups. The first division differentiated between the number of abortions (114 patients with 2 miscarriages and 38 women with at least 3 miscarriages), while the second one between trimester of pregnancy when the miscarriage occurred (123 patients with miscarriages only in the first trimester of pregnancy, <13 gw, and 29 women with miscarriages in the first and second trimester of pregnancy, <21gw).

Control group

The control group included 180 women with a positive history of at least one pregnancy and birth of a healthy newborn at term, and a negative history of miscarriage (mean age 29,46±4,26 years, range 19-42 years, median 29 years). All women with a history of miscarriage, other obstetric complications that may be related to thrombotic changes (preeclampsia, fetal hypotrophy, preterm delivery, preterm placental ablation, intrauterine fetal death), and chronic diseases were excluded from the analysis. Gestational age at delivery was established on the basis of the date of the last menstruation, regularity of periods and ultrasound examinations (Table I).

Genetic analysis

Genomic DNA was extracted from blood leucocytes using QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany). Genotyping was performed using the polymerase chain reaction (PCR) and restriction length fragment polymorphism (RLFP) procedures. The following starters were used for amplification: F 5' – TCG CAT GAT TGC TAT GGG AC-3', R 5'-GTT GAC ATT CCC CAT GGG AC– 3' [10]. PCR products (356 bp - *P0/P0;* 366, 356 bp - *P0/P10;* 366 bp - *P10/P10*) were hydrolyzed with restriction enzyme *Eco130*I (C^CWWGG) to obtain better resolution for visualization. The analysis of the digested fragments was performed by 2% agarose gel electrophoresis. *P0/P0* genotype was identified at the presence of 309, 31, 16 bp, genotype *P0/P10* - 309, 249, 70, 31, 16 bp and genotype *P10/P10* - 249, 70, 31, 16 bp band.

Statistical analyses

Statistical analyses were performed with SPSS17.0 PL for Windows. The *p* value of <0,05 was considered as statistically significant. Frequencies of genotypes were compared by chi-square test (one-sided Fisher test). The expected genotype frequencies were calculated from allele frequencies with the use of the Hardy-Weinberg equation.

Results

The -323P0/P10 factor VII gene polymorphism in the study and control groups

Our research revealed a slightly higher frequency of homozygotic *P0/P0* genotype in the study group (80,26 *vs.* 76,67% in the control group, *p*=0,25). Heterozygotic *P0/P10* genotype was observed more frequently in the control group as compared to patients with a history of miscarriage (18,42 *vs.* 22,78% in the control group, *p*=0,20). The frequency of *P0* allele was comparable in both investigated groups (89,47% in the study group *vs.* 88,06% in the control group, *p*=0,33). A similar situation was observed for the mutated *P10* allele (10,53% in the study group *vs*. 11,94% in the control group, *p*=0,33) (Table II).

Table I. Patient characteristics.

Clinical data	Study group (n=152)	Control group (n=180)	*p*
age (years)			
mean (SD)	31,48±4,17	30,67±3,98	
median	31	30	0,0716
min/max	21-45	19-42	
systolic blood pressure (mmHg)			
mean (SD)	108,52±14,73	110,86±11,25	
median	110	110	0,1021
min/max	80-190	90-150	
diastolic blood pressure (mmHg)			
mean (SD)	68,55±11,65	70,25±9,83	
median	70	70	0,1502
min/max	50-120	50-95	
height (cm)			
mean (SD)	166,26±5,49	166,57±5,90	
median	167	166	0,6228
min/max	150-180	153-180	
body weight (kg)			
mean (SD)	62,97±11,63	61,06±9,36	
median	62	60	0,0983
min/max	43-135	43-105	
BMI (kg/m^2)			
mean (SD)	22,78±4,08	22,00±3,18	
median	22,22	21,23	0,0484
min/max	16,26-47,83	16,73-38,57	
miscarriages			
2	114		
3 and more	38	-	
the term of miscarriage			
I trimester (<13 gw)			
I and II trimester (<21 gw)	123 29	-	
nulliparous multiparous	-	109 71	
gw at delivery			
mean (SD)		39,08±1,20	
median		39	
min/max	-	37-42	
birth weight (g)			
mean (SD)		3472,18±411,97	
median		3470	
min/max		2350-4610	
Ap 1'			
mean (SD)		9,50±1,18	
median		10	
min/max		2-10	
Ap 5'			
mean (SD)		9,93±0,45	
median		10	
min/max	-	5-10	
mode of delivery			
spontaneous		133	
operative	-	47	

Table II. The frequency of genotypes and alleles of -323P0/P10 factor VII gene polymorphism in the study group and in the control group.

-323P0/10	Study group (n=152)		Control group (n=180)		OR	95%CI	p
Genotypes	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)			
P0/P0	122 (80,26)	80,05	138 (76,67)	77,54	1,24	0,73-2,10	0,25
P0/P10	28 (18,42)	18,84	41 (22,78)	21,03	0,77	0,45-1,31	0,20
P10/P10	2 (1,32)	1,11	1 (0,55)	1,43	2,39	0,21-26,58	0,44
Suma	152 (100,00)	100	180 (100,00)	100			
Alleles							
P0	272 (89,47)	—	317 (88,06)	—	1,15	0,71-1,87	0,33
P10	32 (10,53)	—	43 (11,94)	—	0,87	0,53-1,41	0,33
Total	304 (100,0)	—	360 (100,0)	—			

Table III. The frequency of genotypes and alleles of -323P0/P10 factor VII gene polymorphism in the subgroup of women with the history of 2 miscarriages and in the subgroup of women with a history of 3 or more miscarriages.

-323P0/10	Study group (n=152)								Control group (n=180)	
	2 miscarriages (n=114)				3 or more miscarriages (n=38)					
Genotypes	observed value n (%)	expected value (%)	OR	p	observed value n (%)	expected value (%)	OR	p	observed value n (%)	expected value (%)
P0/P0	89 (78,07)	77,72	1,08	0,45	33 (86,84)	87,27	2,01	0,12	138 (76,67)	77,54
P0/P10	23 (20,18)	20,88	0,86	0,35	5 (13,16)	12,29	0,51	0,13	41 (22,78)	21,03
P10/P10	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										
P0	201 (88,16)	—	1,01	0,54	71 (93,42)	—	1,93	0,12	317 (88,06)	—
P10	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 IV. The frequency of genotypes and alleles of -323P0/P10 factor VII gene polymorphism in the subgroup of women with the history of pregnancy losses only in the first trimester and in the subgroup of women with the history of pregnancy losses in the first and second trimester.

-323P0/10	Study group (n=152)								Control group (n=180)	
	I trimester (n=123)				I and II trimester (n=29)					
Genotypes	observed value n (%)	expected value (%)	OR	P	observed value n (%)	expected value (%)	OR	p	observed value n (%)	expected value (%)
P0/P0	100 (81,30)	80,71	1,32	0,21	22 (75,86)	77,32	0,96	0,54	138 (76,67)	77,54
P0/P10	21 (17,07)	18,26	0,70	0,14	7 (24,14)	21,22	1,08	0,52	41 (22,78)	21,03
P10/P10	2 (1,63)	1,03	2,96	0,36	0 (0,00)	1,46	—	0,86	1 (0,55)	1,43
Total	123 (100,0)	100			29 (100,0)	100			180 (100,00)	100
Alleles										
P0	221 (89,84)	—	1,20	0,29	51 (87,93)	—	0,99	0,56	317 (88,06)	—
P10	25 (10,16)	—	0,83	0,29	7 (12,07)	—	1,01	0,56	43 (11,94)	—
Total	246 (100,0)	—			58 (100,0)	—			360 (100,0)	—

*analyzed subgroups were compared to the control group

The -323P0/P10 factor VII gene polymorphism in the subgroup with 2 miscarriages and in the subgroup with 3 or more miscarriages

The frequency of *-323P0/P10* polymorphism genotypes and alleles was similar in the subgroup of women with two miscarriages and in the control group (*P0/P0*: 78,7 *vs*. 76,67%, *p*=0,45; *P0/P10*: 20,18 *vs.* 22,78%, *p*=0,35; *P10/P10*: 1,75 *vs*. 0,55%, *p*=0,33; *P0*: 88,16 *vs.* 88,06%, *p*=0,54; *P10*: 11,84 *vs.* 11,94%, *p*=0,54) (Table III).

In the subgroup of women with 3 or more miscarriages, a higher frequency of homozygotic *P0/P0* genotype in comparison to the control group (86,84 *vs.* 76,67% in controls, *p*=0,12) was observed. Heterozygotic *P0/P10* genotype was also much more frequent in controls (13,16% *vs.* 22,78% in the control group, *p*=0,13). The analysis of the frequency of alleles revealed an overrepresentation of the *P0* allele in the subgroup of women with 3 or more pregnancy losses (93,42 *vs.* 88,06% in the control group, *p*=0.12). Mutated *P10* allele was found more often in the control group (6,58 *vs.* 11,94% in controls, *p*=0,12) (Table III).

The -323P0/P10 factor VII gene polymorphism in the subgroup with pregnancy loss only in the first trimester and in the subgroup with pregnancy loss in the first and second trimester

The frequency of homozygotic *P0/P0* genotype was slightly higher in the subgroup with miscarriage only in the first trimester of pregnancy (81,30 *vs.* 76,67% in the control group, *p*=0,21). An opposite correlation was observed with regard to the heterozygotic *P0/P10* genotype, which was more frequent in the control group (17,07 vs. 22,78% in controls, *p*=0,14). The mutated homozygotic *P10/P10* genotype was present only in 2 patients from the subgroup with miscarriage in the first trimester and in 1 woman from the control group (1,63 *vs.* 0.55% in the control group, *p*=0,36). The frequency of alleles was similar in both investigated groups (*P0*: 89,94 *vs.* 88,06% in controls, *p*=0,29; *P10*: 10,16 *vs.* 11,94% in controls, *p*=0,29) (Tab. IV).

In the subgroup of women with miscarriage in the first and in the second trimester, the frequencies of homozygotic *P0/P0* and heterozygotic *P0/P10* genotypes were similar to those observed in the control group (*P0/P0*: 75,86 *vs.* 76,67% in controls, *p*=0,54; *P0/P10*: 24,14 *vs.* 22,78% in controls, *p*=0,52). The analysis of the allele frequency did not reveal any significant differences between both groups (*P0*: 87,93 *vs.* 88,06% in controls, *p*=0,56; *P10*: 12,07 *vs.* 11,94% in controls, *p*=0,56) (Table IV).

Discussion

Currently, inherited thrombophilia as a possible strong cause of recurrent pregnancy loss remains the subject of much heated debate [1, 2]. Additionally, there is much controversy over tests for inherited thrombophilia and its treatment in pregnant women with history of recurrent miscarriage [11, 12, 13]. Thus, each study of genetic polymorphisms and their contribution to the etiology of miscarriage is an important voice in the debate, especially that, to the best of our knowledge, this has been the first study on the correlation between *-323P0/P10* factor VII gene polymorphism and recurrent miscarriage.

Liu et al., investigated the frequency of *-323P0/P10* and *Arg353Gln* polymorphisms in 209 healthy males and 214 healthy females from the Chinese population. The incidence of the mutated *-323P10* allele was 0.036 and mutated *353Gln* allele was 0.045, respectively. Moreover, they obtained a remarkable confirmation of a strong linkage disequilibrium between the two sites. Also, both of these genetic variants were correlated with serum concentration and activity of factor VII. The authors noted that the influence of *-323P0/P10* polymorphism on gene expression is more excessive than of the *Arg353Gln* genetic variant. Mutated *-323P10* allele correlates with reduced transcription of factor VII gene, what results in decreased factor VII serum level [14].

Our results showed a higher frequency of homozygotic *P0/P0* genotype and lower frequency of heterozygotic *P0/P10* genotype in the study group with recurrent miscarriage as compared to controls. A similar correlation was observed after dividing the study group into subgroups on the basis of the number of miscarriages. These differences were especially visible in the subgroup of women with 3 or more pregnancy losses. Also, in this subgroup of women, a lower frequency of the mutated *-323P10* allele was observed (-323*P0/P10:* 13,16 *vs.* 22,78% in controls, OR=0,51, ns, P10: 6,58 *vs.* 11,94% in controls, OR=0,52, ns).

We assumed that *-323P0/P10* factor VII gene polymorphism may influence the coagulation changes and, consequently, be a risk factor of recurrent miscarriage. This hypothesis was based on other studies on the *-323P0/P10* genetic variant and its correlation with the development of cardio-vascular diseases conditioned by thrombotic changes. What is remarkable, conclusions of these researches are conflicting. Some of these studies confirm the existence of a correlation between *-323P10* allele and an increased risk of thrombotic changes, while others report the opposite, protective role of *-323P10* allele with regard to the risk of coronary artery diseases [9, 15, 16, 17].

Mo et al., performed a meta-analysis of the relationship between factor VII gene polymorphisms, among others *-323P0/P10* genetic variant, and the risk of coronary artery diseases in various populations. The meta-analysis involved 39 studies, out of which 12 were focused on the *-323P0/P10* polymorphism. The myocardial infarction risk was estimated in 2862 patients and 4240 controls. Finally, the meta-analysis confirmed an important role of the mutated *-323P10* allele in the etiology of cardio-vascular diseases [9].

The study of Ben-Hadji-Khalifa et al., also proved the relationship between the *-323P0/P10* factor VII gene polymorphism and the risk of thrombotic changes. The study group consisted of 308 patients with acute cardio-vascular diseases, whereas 312 healthy subjects were enrolled in the control group. The factor VII serum concentration level was statistically significantly higher in the study group. Moreover, this concentration was positively correlated with the severity of the disease. The genetic analysis revealed that carriers of *-323P10* allele were in the group of increased risk for acute cardio-vascular diseases, but this genetic variant did not change the factor VII serum concentration level [15].

The aim of the study by Sakowicz et al., was to evaluate the correlation of the *-323P0/P10* polymorphism with the risk for myocardial infarction in patients below 45 years of age, in a Polish population. The study group comprised 266 patients below 45 years of age with a positive history of myocardial infarction. The control group consisted of 137 healthy subjects > 45 years of age. A decreased risk of myocardial infarction was noted in the carriers of the mutated *-323P10* allele. Their study confirmed

a correlation between the *-323P0/P10* genetic variant and thrombotic incidents, but the conclusions are not in agreement with observations of other researchers. Sakowicz et al., showed that the presence of mutated *-323P10* allele increases the risk of thrombotic changes. They demonstrated a protective role of *-323P10* allele against the risk of myocardial infarction [16].

Similar observations were made by Huang et al. Their study was performed in a group of 1020 patients with coronary artery diseases confirmed by an angiographic examination. The control group consisted of 1112 healthy volunteers. Genetic analysis investigated the frequency of genotypes and alleles of *-323P0/P10, R353Q* and *HVR4* factor VII gene polymorphism. The obtained results confirmed the protective role of *-323P10* allele in the etiology of coronary artery diseases [17].

Conclusions

The analysis of *-323P0/P10* factor VII polymorphism revealed a probable protective role of mutated *-323P10* allele against the risk of recurrent miscarriage in women with 3 or more pregnancy losses. A comprehensive research in a larger sample size is needed to confirm the observed correlation. Moreover, influence of other genetic and environmental factors ought to be taken into consideration.

Oświadczenie autorów:

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