The contribution of Hind III C>G PAI-1 gene polymorphism in etiology of recurrent miscarriages

Udział polimorfizmu Hind III C>G genu PAI-1 w etiologii poronień nawracających

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Abstract

Objectives: *The goal of the study was to assess the relationship of HindIII C>G PAI-1 gene polymorphism with increased risk of recurrent miscarriages.*

Material and methods: *A whole of 152 women with a history of at least two miscarriages were classified into analysis. The study group was divided twice (114 subjects with 2 miscarriages and 38 subjects with >3 miscarriages, 123 subjects with miscarriages at <13gw, and 29 subjects with miscarriages in <21gw). The controls consisted of 180 women with a positive history of at least one pregnancy and birth of a healthy term newborn, and a negative history of miscarriage. The analysed polymorphisms were determined by PCR/RFLP methods.*

Results: *The occurrence of HindIII GG genotype in the whole study group was 25.7% and 20.0% in controls (OR=1.38, p=0.14). HindIII G allele was also observed more frequently in the whole study group (45.7% vs. 42.2% in controls, OR=1.15, p=0.20). The occurrence of HindIII GG genotype was higher in the subgroup of women with >3 miscarriages (31.6% vs. 20.0% in controls, OR=1.85, p=0.09). HindIII G allele was also noted more frequently in the subgroup of women with >3 miscarriages (50.0% vs. 42.2% in controls, OR=1.37, p=0.13). A tendency of higher frequency of HindIII GG genotype and HindIII G allele was also noted in the subgroup of patients with miscarriages in the first and second trimester (HindIII GG: 31.0% vs. 20.0% in controls, OR=1.80, p=0.14, HindIII G: 51.7% vs. 42.2% in controls, OR=1.47, p=0.11).*

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Conclusions: *Mutated HindIII G allele and HindIII GG genotype of HindIII C>G polymorphism probably augment the risk of recurrent miscarriages.*

Key words: **recurrent miscarriages** / **PAI-1** /**gene polymorphism** /

Streszczenie

Cel pracy: *Ocena związku polimorfizmu HindIII C>G genu PAI-1 z występowaniem poronień nawracających.*

Metody: *W badaniu uwzględniono dwie grupy kobiet: 152 pacjentki z obciążonym wywiadem w kierunku występowania poronień (dwóch lub więcej) oraz 180 pacjentek, u których potwierdzono w wywiadzie co najmniej jedną ciążę o niepowikłanym przebiegu, zakończoną urodzeniem zdrowego, donoszonego noworodka oraz wykluczono wystąpienie poronień. W grupie kobiet z poronieniami nawracającymi dokonano podziału na podgrupy (114 kobiet z dwoma poronieniami w wywiadzie oraz 38 kobiet z >3 poronieniami w wywiadzie, 123 pacjentki, u których poronienia występowały poniżej 13 tc. oraz 29 kobiet, u których poronienia miały miejsce poniżej 21 tc.). Analizę genetyczną przeprowadzono przy zastosowaniu metody PCR/RFLP.*

Wyniki: *Zmutowany genotyp HindIII GG w grupie badanej występował z częstością 25,7%, natomiast wśród kobiet z grupy kontrolnej z częstością 20,0% (OR=1,38, p=0,14). Podobna obserwacja dotyczyła częstości występowania zmutowanego allela HindIII G (45,7% vs. 42,2% w grupie kontrolnej, OR=1,15, p=0,20). W podgrupie pacjentek z >3 poronieniami frekwencja genotypu HindIII GG była wyraźnie wyższa niż w grupie kontrolnej (31,6% vs. 20,0% w grupie kontrolnej, OR=1,85, p=0,09). Również frekwencja allela HindIII G była wyższa w tej podgrupie (50,0% vs. 42,2% w grupie kontrolnej, OR=1,37, p=0,13). Tendencja do częstszego występowania genotypu HindIII GG i allela HindIII G była także obserwowana u pacjentek z poronieniami <21 tc (odpowiednio: HindIII GG: 31,0% vs. 20,0% w grupie kontrolnej, OR=1,80, p=0,14, HindIII G: 51,7% vs. 42,2% w grupie kontrolnej, OR=1,47, p=0,11).*

Wnioski: *Zmutowany allel HindIII G oraz zmutowany genotyp homozygotyczny HindIII GG polimorfizmu HindIII C>G genu PAI-1 prawdopodobnie zwiększają ryzyko występowania poronień nawracających.*

Słowa kluczowe: **poronienia nawracające** / **PAI-1** / **polimorfizm genetyczny** /

Introduction

Plasminogen activator inhibitor – 1 (PAI-1) belongs to the family of serine proteinase inhibitors and is the most important natural inhibitor of tissue plasminogen activator and urokinase plasminogen activator, thus playing a vital role in the regulation of fibrinolysis processes. Changes in the PAI-1 serum concentration and activity, which may be genetically conditioned, may result in hypofibrinolysis, thrombotic changes in the utero-placental unit and increased risk of recurrent pregnancy loss, preeclampsia, gestational hypertension, intrauterine growth restriction, or intrauterine fetal death [1, 2].

PAI-1 is synthetized mainly in the endothelial cells, hepatocytes and vessel smooth muscle cells. Enhanced expression of *PAI-1* gene, besides thrombophilia, may lead to cell apoptosis resistance. It is probably mediated by reduction of cellular adhesion and influence on intracellular signalization. PAI-1 is also involved in adipose tissue development and control of insulin activity in adipocytes. Increased PAI-1 expression is observed in obese patients. There is a positive correlation between PAI-1 activity and body mass index, the amount of visceral adipose tissue, serum concentration of insulin, glucose and triglycerides. Gene encoding PAI-1 (7q21,3-22) consists of 9 exons and 8 introns. *PAI-1* gene expression is enhanced by endothelial growth factor, steroids, estrogens, insulin, cytokines, bacterial endotoxins, thrombine, LDL cholesterol, prostaglandins, and fatty acids [3, 4].

HindIII C>G PAI-1 gene polymorphism is localized in 3'UTR region (untranslated region) and concerns substitution of cytosine into guanine. It is believed that the presence of *HindIII G* allele disturbs proper regulation of PAI-1 gene translation, what leads to increased concentration of PAI-1 in serum and development of prothrombotic state [5]. This genetic variant is correlated with increased risk of thromboembolic disease, coronary heart disease, preeclampsia, and idiopathic pulmonary hypertension. There is a connection between PAI-1 serum concentration and LDL cholesterol and insulin concentration. The study of Dawson et al., suggests that this regulation is being caused by the presence of *HindIII C>G* polymorphism [6]. Research of De la Cruz-Mosso et al., revealed that *HindIII CG* and *HindIII GG* genotypes influence the concentrations of total cholesterol [5]. The involvement of this genetic variant is considered in susceptibility to systemic lupus erythematosus or increased PAI-1 concentration in patients with rheumatoid arthritis [7, 8].

Objectives

The aim of the study was to evaluate the correlation of *HindIII C>G PAI-1* gene polymorphism with increased risk of recurrent miscarriages. Our study hypothesis is based on the fact that PAI-1 plays a central role in the process of fibrinolysis and any changes in PAI-1 concentrations and activity may cause thrombotic changes in the utero-placental unit.

Material and methods

All subjects (Caucasian, of Polish origin) were enrolled in the study at the Department of Perinatology and Women's Diseases, Poznan University of Medical Sciences between 2011-2013. Each woman gave her written confirmed consent to participate in the research. The project was approved by the Local Bioethics Committee (nr 422/11).

A total of 152 (mean age 30.16±3.82 years, range 21-45 years, median 30 years) women with a positive history of at least two miscarriages (which was defined as the loss of pregnancy before the end of the 22gw) were enrolled in the study group. Inclusion and exclusion criteria are presented in Table I.

The study group was divided twice: by the number of abortions (114 patients with 2 miscarriages and 38 women with at least 3 miscarriages), and by gestational age at miscarriage (123 patients with miscarriages in the first trimester at <13gw, and 29 women with miscarriages in the first and second trimester of pregnancy at <21gw).

The control group consisted of 180 women with a positive history of at least one pregnancy and birth of a healthy term newborn, and a negative history of miscarriage (mean age 29.46±4.26 years, range 19-42 years, median 29 years). Inclusion and exclusion criteria are presented in Table I.

Genotyping of the *HindIII C>G PAI-1* gene polymorphism was performed in each woman from the study and the control group. 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). The following starters were used for amplification: F 5' -gCC TCC AgC TAC CgT TAT TgT ACA -3', R 5'- CAg CCT AAA CAA CAg AgA CCC C -3' [9]. PCR product (754 bp) was hydrolyzed with restriction enzyme *Hind*III (A^AGCTT) (*Hind III CC* – 566, 188 bp, *HindIII CG* – 754, 566, 188 bp, *HindIII GG* – 754 bp). The analysis of the digested fragments was performed by 2% agarose gel electrophoresis. Statistical analyses were performed with SPSS17.0 PL for Windows. 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 HindIII C>G PAI-1 gene polymorphism in the study and control groups

The frequency of *HindIII CC* genotype was similar in both analyzed groups (34.2% *vs.* 35.6% in controls, OR=0.94, *p*=0.44). There was slight overrepresentation of the *HindIII CG* genotype in the control group as compared to the group of women with miscarriages (44.4% in controls *vs.* 40.1%, OR=0.84, *p*=0.25). Interestingly, the frequency of the mutated *HindIII GG* genotype was higher in the study group (25.7% *vs*. 20.0% in controls, OR=1.38, *p*=0.14). The study revealed that *HindIII C* allele was present more often in the control group (57.8% in controls *vs.* 54.3%, OR=0.87, *p*=0.20). Another correlation was observed with regard to the mutated *HindIII G* allele, which was more frequent in the group of women with a positive history of miscarriages (45.7% *vs.* 42.2% in controls, OR=1.15, *p*=0.20) (Table II).

The HindIII C>G PAI-1 gene polymorphism in the subgroup with 2 miscarriages and in the subgroup with 3 or more miscarriages

The study revealed no significant differences between the subgroup of women with 2 miscarriages and the control group (*HindIII CC*: 35.5% *vs.* 35.6%, *p*=0.51; *HindIII CG*: 41.2% *vs.* 44.4%, *p*=0.34; *HindIII GG*: 23.7% *vs.* 20.0%, *p*=0.27; *HindIII C*: 55.7% *vs.* 57.8%, *p*=0.34; *HindIII G*: 44.3% *vs.* 42.2%, *p*=0.34) (Table III).

As far as the subgroup of women with 3 or more miscarriages is concerned, lower frequency of the *HindIII CC* genotype in comparison to the control group was observed (35.6% in controls *vs.* 31.6%, OR=0.84, *p*=0,39). The *HindIII CG* genotype was also present more often in the control group (44.4% in controls *vs.* 36.8%, OR=0.73, *p*=0.25). Interestingly, the frequency of the mutated *HindIII GG* genotype was higher in the subgroup of patients with at least 3 pregnancy losses as compared to controls (31.6% *vs*. 20.0%, OR=1.85, *p*=0.09). The *HindIII C* allele was also more frequently observed in the control group (50.0% *vs.* 57.8% in controls, OR=0.73, *p*=0.13), whereas the *HindIII G* allele was more often detected in the subgroup of women with 3 or more miscarriages (50.0% *vs.* 42.2% in controls, OR=1.37, *p*=0.13) (Table III).

The HindIII C>G PAI-1 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 analysis revealed that the frequency of the *HindIII CC* genotype was similar in the subgroup of patients with pregnancy loss only in the first trimester and in controls (35.8% *vs.* 35.6% in controls, *p*=0.53). The *HindIII CG* genotype was observed more often in the control group as compared to the subgroup with miscarriages at <13 gw (44.4% in controls *vs.* 39.8%, *p*=0.25). An opposite correlation concerned the *HindIII GG* genotype, which was more frequent in the subgroup of patients with miscarriages in the first trimester (24.4% *vs.* 20.0% in controls, *p*=0.22). The frequency of alleles was similar between both analyzed groups (*HindIII C*: 55.7% *vs.* 57.8% in controls, *p*=0.33; *HindIII G*: 44.3% vs. 42.2% in controls, *p*=0.33) (Table IV).

In the subgroup of women with pregnancy loss in the first and second trimester, the frequency of the *HindIII CC* genotype was lower than in the control group (27.6% *vs.* 35.6% in controls, OR=0.69, *p*=0.27), whereas the observed frequency of the *HindIII CG* genotype was similar in both analyzed groups (41.4% *vs.* 44.4% in controls, OR=0.88, *p*=0.46). Noteworthy, *HindIII GG* occurred more often in the subgroup of women with pregnancy loss in the first and second trimester (31.0% *vs.* 20.0%, in controls, OR=1.80, *p*=0.14). The *HindIII C* allele was observed more often in the control group (57.8% in controls *vs.* 48.3%, *p*=0.11), and the *HindIII G* allele – in the subgroup of women with pregnancy loss in the first and second trimester (51.7% *vs.* 42.2% in controls, OR=1.47, *p*=0.11) (Table IV).

Discussion

Proper PAI-1activity is essential to maintain pregnancy. PAI-1 genetic variants are often involved in studies concerning obstetrical complications. Beside recurrent miscarriages, they are probably involved in preeclampsia, gestational hypertension,

Table I. Inclusion and exclusion criteria to the study and the control group.

	Inclusion criteria	*Exclusion criteria*
Study group (n=152)	- positive history of two or more miscarriages	- known reasons for miscarriage (anatomical anomalies of genito-urinary tracts, chromosomal defects, chronic diseases, infections, hormonal impairments, antiphospholipid syndrome) - cervical insufficiency - thromb-embolic disease - other obstetric complications that could be a reason for miscarriage (e.g. gestational hypertension, eclampsia, preeclampsia, gestational diabetes)
Control group (n=180)	- positive history of at least one pregnancy and birth of a healthy newborn at term	- positive history of miscarriage - positive history of other obstetric complications that may be related to thrombotic changes (preeclampsia, fetal hypotrophy, preterm delivery, preterm placental ablation, intrauterine fetal death) - presence of chronic diseases and thromb-embolic disease

Table II. The frequency of genotypes and alleles of HindIII C>G PAI-1 gene polymorphism in the whole study group and in the control group.

HindIII C>G	*Study group (n=152)*		*Control group (n=180)*		*OR*	*95%CI*	*p*
Genotypes	***Observed value n (%)***	***Expected value (%)***	***Observed value n (%)***	***Expected value (%)***			
CC	52 (34,2)	29,5	64 (35,6)	33,4	0,94	0,59-1,48	0,44
CG	61 (40,1)	49,6	80 (44,4)	48,8	0,84	0,54-1,30	0,25
GG	39 (25,7)	20,9	36 (20,0)	17,8	1,38	0,82-2,31	0,14
Total	152 (100,0)	100	180 (100,0)	100			
Alleles							
C	165 (54,3)	—	208 (57,8)	—	0,87	0,64-1,18	0,20
G	139 (45,7)	—	152 (42,2)	—	1,15	0,85-1,57	0,20
Total	304 (100,0)	—	360 (100,0)	—			

intrauterine growth restriction, or intrauterine fetal death. It is believed that one of the mechanisms leading to those failures are hemostasis changes resulting in thrombophilia. Co-existence of genetically conditioned thrombophilia and physiological pro-thrombotic state during pregnancy may lead to impairments of the utero-placental unit. Moreover, enhanced PAI-1 secretion by endothelial cells results in forming micro-clots in spiral arteries and reduction of placental blood flow. It is clearly shown in studies concerning increased PAI-1 concentration in patients with preeclampsia [2, 9, 10]. The fact of already proven correlation between recurrent pregnancy loss and genetic variants of other factors of hemostasis cascade, e.g. factor V Leiden, factor VII or prothrombin is especially noteworthy [11, 12, 13].

Functional *-675 4G/5G* polymorphism is one of the most often analyzed PAI-1 genetic variants. The presence of the *-675 4G* allele is correlated with enhanced *PAI-1* gene transcription, increased PAI-1 concentration, and increased prothrombotic activity. Involvement of the *-675 4G/5G* polymorphism is suggested in intrauterine fetal death, preterm placental abruption, intrauterine growth restriction, recurrent miscarriages, and preeclampsia [10, 14, 15].

Jeon et al., evaluated the association of selected *PAI-1* gene polymorphisms *(-844G>A, -675 4G/5G, 43G>A, 9785G>A, 11053T>G*) with idiopathic recurrent pregnancy loss in Korean women. A total of 308 patients were enrolled in the study group. The control group consisted of 227 women. The study revealed that the *4G4G* and *-844AA/4G4G/11053GG* genotypes were correlated with an increased risk of recurrent miscarriages and that the *-844A/4G/43G/9785G/11053G* haplotype was associated the hypofibrinolytic status (i.e. increased levels of plasma PAI-1, increased numbers of platelets, reduced prothrombin time, and reduced activated partial thromboplastin time). Moreover, the obtained results suggested a positive correlation between the presence of PAI-1 *11053TG+GG* genotypes with plasma concentration of homocysteine and urate. These authors concluded that *PAI-1 -844G>A, 4G/5G*, and *11053T>G* polymorphisms are risk factors or recurrent pregnancy loss [16].

The aim of the study of Magdoud et al., was to investigate the association between the *PAI-1 -844G/A* and *4G/5G (-675G/A)* polymorphisms with the risk of recurrent pregnancy loss. The analysis involved 304 women with recurrent miscarriages and 371 controls. Their study revealed that minor allele frequency of

Table III. The frequency of genotypes and alleles of HindIII C>G PAI-1 gene polymorphism in the subgroup of women with 2 miscarriages, 3 or more miscarriages and in the control group.

HindIII C>G	*Study group (n=152)*								*Control group (n=180)*	
	2 miscarriages (n=114)				*≥3 miscarriages (n=38)*					
Genotypes	*Observed value n (%)*	*Expected value (%)*	*OR*	*p*	*Observed value n (%)*	*Expected value (%)*	*OR*	*p*	*Observed value n (%)*	*Expected value (%)*
CC	40 (35,1)	31,0	0,98	0,51	12 (31,6)	25,0	0,84	0,39	64 (35,6)	33,4
CG	47 (41,2)	49,4	0,88	0,34	14 (36,8)	50,0	0,73	0,25	80 (44,4)	48,8
GG	27 (23,7)	19,6	1,24	0,27	12 (31,6)	25,0	1,85	0,09	36 (20,0)	17,8
Total	114 (100,0)	100,0			38 (100,0)				180 (100,0)	100
Alleles										
C	127 (55,7)	–	0,92	0,34	38 (50,0)	–	0,73	0,13	208 (57,8)	–
G	101 (44,3)	–	1,09	0,34	38 (50,0)	–	1,37	0,13	152 (42,2)	–
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 HindIII C>G PAI-1 gene polymorphism in the subgroup of women with miscarriages only in the first trimester, in the subgroup with pregnancy loss in the first and second trimester and in the control group.

HindIII C>G	*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 (%)*
CC	44 (35,8)	31,0	1,01	0,53	8 (27,6)	23,3	0,69	0,27	64 (35,6)	33,4
CG	49 (39,8)	49,4	0,83	0,25	12 (41,4)	49,9	0,88	0,46	80 (44,4)	48,8
GG	30 (24,4)	19,6	1,29	0,22	9 (31,0)	26,8	1,80	0,14	36 (20,0)	17,8
Total	123 (100,0)	100				100			180 (100,0)	100
Alleles										
C	137 (55,7)	–	0,92	0,33	28 (48,3)	–	0,68	0,11	208 (57,8)	–
G	109 (44,3)	–	1,09	0,33	30 (51,7)	–	1,47	0,11	152 (42,2)	–
Total	246 (100,0)	–			58 (100,0)	–			360 (100,0)	–

*analyzed subgroups were compared to the control group

4G/5G ($p < 0.001$), but not *-844G/A* ($p = 0.507$), was higher in the study group [17].

The analysis of Torabi et al., whose research involved 100 women with a positive history of at least two consecutive spontaneous abortions and 100 controls (positive history of at least two live births and a negative history of miscarriages), is also most interesting. Each subject was evaluated for the presence of 11 thrombophilic gene polymorphisms (factor V Leiden, factor V *4070 A/G*, factor V *5279 A/G*, factor XIII *103 G/T*, factor XIII *614 A/T*, factor XIII *1694 C/T*, PAI-1 *-675 4G/5G*, ITGB3 *1565 T/C*, β-fibrinogen *-455G/A*, MTHFR *677 C/T*, MTHFR *1298 A/C*). The obtained results confirmed an association of only six from eleven of the above polymorphisms with increased risk of recurrent miscarriages (factor V *1691G/A*, factor V *5279A/G*, factor XIII *614A/T*, β-fibrinogen *-455G/A*, ITGB3 *1565T/C*, and MTHFR *1298A/ C*) [18].

To the best of our knowledge, this has been the first study on the correlation between the *HindIII C>G PAI-1* gene polymorphism and recurrent miscarriage. Despite lack of statistically significant differences as to the frequency of genotypes and alleles between the analyzed groups, attention was paid to the more frequent occurrence of the *HindIII GG* genotype and the *HindIII G* allele in the study group. This correlation is much more clear in the subgroup of women with 3 or more pregnancy losses. Moreover, a tendency for higher frequency of the *HindIII GG* genotype and the *HindIII G* allele was also noted in the subgroup of women with miscarriages in the first and second trimester.

Conclusions

The obtained results suggest that the mutated *HindIII G* allele and mutated homozygotic *HindIII GG* genotype of *HindIII C>G PAI-1* gene polymorphism probably increase the risk of recurrent miscarriages. This hypothesis is also supported by some evidence on the correlation of the *HindIII G* allele with impairments of proper PAI-1 gene translation regulation, what results in increased PAI-concentration [5].

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