Ginekol Pol. 2016, 87, 271-276

DOI: 10.17772/gp/62203

PRACE ORYGINALNE

położnictwo

Coexistence of ACE (I/D) and PAI-1(4G/5G) gene variants in recurrent miscarriage in Polish population

Współwystępowanie wariantów polimorficznych genów ACE (I/D) i PAI-1 (4G/5G) w poronieniach nawracających w populacji polskiej

Grażyna Kurzawińska[1,2], Magdalena Barlik[1,2], Krzysztof Drews[1,2], Agata Różycka[1,2], Agnieszka Seremak-Mrozikiewicz[1,2,3], Marcin Ożarowski[3,4], Andrzej Klejewski[5,6], Bogusław Czerny[7,8], Hubert Wolski[1,9]

[1] Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences
[2] Laboratory of Molecular Biology in the Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences
[3] Department of Pharmacology and Phytochemistry, Institute of Natural Fibers and Medicinal Plants, Poznan, Poland
[4] Department of Pharmaceutical Botany and Plant Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[5] Department of Nursing, Poznan University of Medical Sciences, Poznan, Poland
[6] Department of Obstetrics and Women's Diseases, Poznan University of Medical Sciences, Poznan, Poland
[7] Department of General Pharmacology and Pharmacoeconomics, Pomeranian Medical University, Szczecin, Poland
[8] Department of Stem Cells and Regenerative Medicine, Institute of Natural Fibres and Medicinal Plants, Poznan, Poland
[9] Division of Gynecology and Obstetrics, Podhale Multidisciplinary Hospital, Nowy Targ, Poland

Abstract

Objectives: *Recurrent miscarriage (RM) is one of the most common obstetric complications. Numerous studies have suggested that genetic variants leading to an impaired balance between coagulation and fibrinolysis may contribute to elevated risk of pregnancy loss. The aim of the study was to investigate a possible association between angiotensin-converting enzyme (ACE, rs1799752) I/D and plasminogen activator inhibitor type 1 (PAI-1, rs1799768) 4G/5G polymorphisms with RM among Polish women.*

Material and methods: *DNA was extracted from peripheral blood samples of 152 women with a history of ≥2 consecutive pregnancy losses before 22 weeks of gestation, and 180 healthy controls with at least 1 live birth at term and no history of pregnancy loss. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were used to identify the polymorphisms.*

Results: *No statistically significant differences were found in genotype and allele frequencies of the studied polymorphisms. The most relevant difference between the study group and controls was found for the ID genotype distribution of the ACE gene (52.6 vs. 46.7%, OR=1.27, p=0.28). The analysis of genotype coexistence revealed a higher incidence of the combination of the ACE II and the PAI-1 4G/4G genotypes in the control group (10.0 vs. 5.9% in control group; p=0.17).*

Conclusions: *The obtained results suggest no apparent association between the ACE I/D, PAI-1 4G/5G polymorphisms and increased RM susceptibility in the analyzed Polish population.*

Key words: **renin-angiotensin system** / **polymorphism** / **PAI-1** / **ACE** / **miscarriage** / / **SERPINE-1** /

Corresponding author:
Grażyna Kurzwińska
Pracownia Biologii Molekularnej w Klinice Perinatologii i Chorób Kobiecych
Uniwersytet Medyczny w Poznaniu, 60-535 Poznań, ul. Polna 33.
tel. 61/8419-613, fax. 61/851-46-47, e-mail: gene@gpsk.am.poznan.pl

Otrzymano: **10.12.2015**
Zaakceptowano do druku: **15.03.2016**

DOI: 10.17772/gp/62203
Ginekol Pol. 2016, 87, 271-276

Streszczenie

Cel pracy: *Poronienia nawracające są jednym z najczęściej występujących powikłań położniczych. Wiele badań wskazuje, że warianty genetyczne prowadzące do zaburzenia równowagi pomiędzy układami krzepnięcia i fibrynolizy mogą powodować wzrost ryzyka utraty ciąży. Celem pracy było zbadanie związku pomiędzy występowaniem poronień nawracających a polimorfizmami I/D genu konwertazy angiotensyny I (ACE, rs1799752) i inhibitora aktywatora plazminogenu typu 1 (PAI-1, rs1799768) 4G/5G wśród kobiet w Polsce.*

Materiał i metody: *DNA izolowano z krwi obwodowej 152 kobiet z dwoma lub więcej następującymi po sobie poronieniami przed 22 tygodniem ciąży i 180 zdrowych kobiet bez poronień, z co najmniej jedną ciążą zakończoną urodzeniem zdrowego noworodka o czasie w wywiadzie. Do identyfikacji polimorfizmów zastosowano łańcuchową reakcję polimerazy (PCR) oraz polimorfizm długości fragmentów restrykcyjnych (RFLP).*

Wyniki: *Nie zaobserwowano statystycznie istotnych różnic w częstości występowania genotypów i alleli badanych polimorfizmów. Największa różnica pomiędzy grupą badaną i kontrolną dotyczyła częstości występowania genotypu ID genu ACE (52,6 vs. 46,7%, WR=1,27, p=0,28). Analiza współwystępowania badanych w pracy genotypów wykazała częstsze występowanie w grupie kontrolnej kombinacji genotypów ACE II oraz PAI-1 4G/4G (10,0 vs.5,9% w grupie kontrolnej; p=0,17).*

Wnioski: *Wyniki wskazują na brak bezpośredniego związku polimorfizmów ACE I/D, PAI-1 4G/5G ze zwiększoną częstością występowania poronień nawracających w badanej populacji polskiej.*

Słowa kluczowe: **układ renina-angiotensyna** / **polimorfizm** / **PAI-1** /**ACE** / **poronienie** /
/ **SERPINE-1** /

Introduction

Recurrent miscarriage (RM) has been defined as the loss of ≥3 consecutive pregnancies before 22 weeks of gestation. The experts from the Polish Gynecological Society recommend to expand the diagnosis of RM and include 2 consecutive spontaneous abortions. There are many well-known risk factors for RM (anatomic, endocrine, immunologic, infectious, biochemical, environmental and some gene variants), and the etiology of this pregnancy complication is believed to be multifactorial. The coagulation system during pregnancy undergoes significant changes, leading to hypercoagulability [1, 2]. Each factor causing an imbalance *between coagulation* and fibrinolysis in pregnancy may cause thrombosis and, consequently, recurrent abortion [3]. A number of epidemiological and experimental studies have demonstrated the existence of links between the fibrinolytic system and the renin-angiotensin system (RAS). RAS is an endocrine system which regulates blood pressure and electrolyte balance. In addition, local expression of the renin-angiotensin system has been found in a number of tissues, including human placenta. The fetal-maternal interface comprises both, fetal placental tissue RAS and maternal decidual tissue RAS [4]. It has been suggested that placental RAS is involved in a few physiological processes (placental angiogenesis, proliferation, and trophoblast invasion) and participates in the etiology of some obstetric complications, e.g. preeclampsia [5, 6]. The key component of RAS is angiotensin-converting enzyme (ACE; EC 3.4.15.1), which is responsible for inactivation of vasodilator bradykinin and conversion of the inactive decapeptide, angiotensin I, to the active octapeptide – angiotensin II. The *ACE* gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. There is a genetic variation within the gene, a 278 base pair *Alu* sequence insertion/deletion (*I/D*) polymorphism (rs1799752). This variant is located in intron 16 and it does not affect the structure of the enzyme, but is strongly connected to the plasma level of ACE (*D allele* is associated with higher enzyme levels than the presence of the *I* allele). The *ACE DD* genotype has been shown to be associated with enhanced conversion of angiotensin I to angiotensin II, which increases the synthesis of plasminogen activator inhibitor -1. Type I plasminogen activator inhibitor (PAI-1) is the predominant inhibitor of both, tissue- and urinary-type plasminogen activators (t-PA, u-PA). The *PAI-1* (SERPINE1) gene is located at 7q21.3-22 and contains 9 exons and 8 introns. The 1-bp guanine deletion/insertion (*4G/5G*, rs1799889) polymorphism at position -675 nucleotides relative to the transcription start site is the commonly studied functional variant in the *PAI-1* gene [7]. The *4G* allele is associated with higher plasma PAI-1 transcription and activity than *5G*. Figure 1 shows the relation between the *ACE* and *PAI-1* polymorphisms.

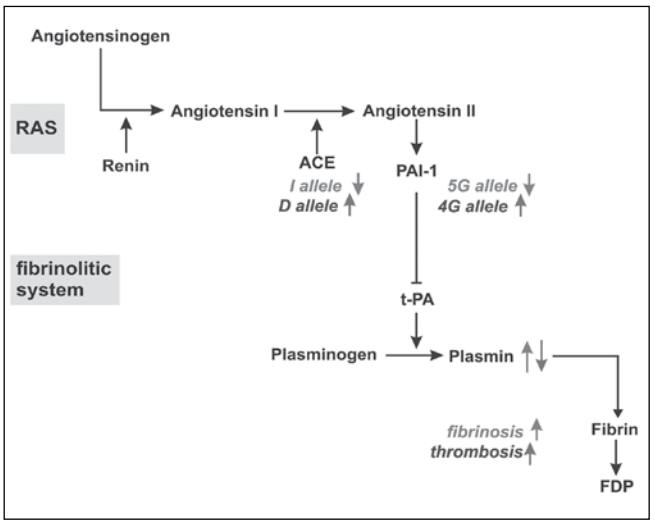

Figure 1. Interaction between fibrinolysis and RAS, including the role of the *ACE* and *PAI-1* polymorphisms.

A number of studies on the association between RM and both, *ACE I/D* and *PAI-1 4G/5G* polymorphisms, together and individually, have been conducted but the results remain inconclusive [8, 9].

Therefore, the aim of the present study was to investigate the effects of the polymorphisms in the *ACE* and *PAI-1* genes from the renin-angiotensin and fibrinolytic systems on the etiology of RM in Polish women from the Wielkopolska region.

Material and methods

A total of 332 women were enrolled at the Department of Perinatology and Women's Diseases, Poznan University of Medical Sciences. All patients signed their informed consent. Local Ethics Committee approved of the study. The women were subdivided into two groups: 152 (mean age 30.16±3.82 years, range 21-45 years, median 30 years) with a positive history of at least 2 consecutive pregnancy losses in the first and second trimester, and 180 (mean age 29.46±4.26 years, range 19-42 years, median 29 years) with a negative history of miscarriage and at least one uncomplicated pregnancy and term delivery. All women were Caucasian and from the Wielkopolska region, Poland.

In each case of a miscarriage, the pregnancy had been previously confirmed by an ultrasound or histopathologic examination performed after pregnancy loss. The exclusion criteria were as follows:

- antiphospholipid antibodies and other pathologies which may be associated with the occurrence of thrombotic complications and miscarriage (chronic hypertension, positive history of deep venous thromboembolism),
- known causes of RM (chromosomal disorders, uterine defects, chronic diseases, infections, hormonal disorders),
- isthmic-cervical insufficiency.

Genomic DNA was extracted from whole blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany), according to the manufacturer's recommendations. Genotyping was performed at the Laboratory of Molecular Biology, Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, using the PCR method for the *ACE I/D* polymorphism, as described previously [10]. The *-675 4G/5G* polymorphism in the *PAI-1* promoter region was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method using the primers and the restriction enzyme *Bse*LI (ThermoScientific, USA) published by Doggen et. al [11]. The products were analyzed by electrophoresis on a 3% agarose gel with Midori Green Advanced DNA Stain (Nippon Genetics, Europe GmbH).

Statistical analysis

All statistical analyses were performed using the R language (version 3.1.0, http://cran.r-project.org), package MCPerm [12]. Distribution of the genotypes in both groups was tested for deviation from the Hardy-Weinberg equilibrium by Chi-square analysis. Differences in allele and genotype frequencies between the groups were evaluated by standard Chi-square tests. The odds ratio (OR) and 95% confidence intervals (95% CI) were also determined. The p-value of <0.05 was considered as statistically significant. The study polymorphisms were also tested for any association with RM using the Cochran-Armitage trend test (CATT).

Results

Distribution of the *PAI-1* and *ACE* allele and genotype frequency among the RM patients and controls is shown in Table I.

No statistically significant differences for the *-675 4G/5G PAI-1* polymorphism between genotype frequencies were found in RM women and controls (*4G/4G* 32.9% *vs.* 35.0%; *4G/5G* 49.3% *vs.* 47.2%, and *5G/5G* was the same in both groups - 17.8%). Values of the observed genotype frequencies were consistent with the expected results, calculated using the Hardy-Weinberg equilibrium (study group $\chi^2 = 0.01$; $p = 0.90$; control group $\chi^2 = 0.13$; $p = 0.72$). Allele frequencies for this polymorphism were: *4G* (57.6% *vs.* 58.6% in the control group, $p = 0.79$), *5G* (42.4% *vs.* 41.4% and the control group, $p = 0.79$) (Table I).

No statistically significant differences were observed in the *ACE* allele and genotype frequency distribution between RM women and controls. Moreover, there were no deviations from the Hardy-Weinberg equilibrium in the investigated groups (RM group χ^2=0.47; p=0.49 and χ^2=0.76; p=0.38 in the control group). The frequency of the *ACE* genotype *II* with insertion was 21.1 *vs*. 24.4% in the control group. The most relevant difference between the study group and controls was found for the *ID* genotype distribution of the *ACE* gene (52.6 *vs*. 46.7%, OR=1.27, p=0.28).

The cross-tabulation of genotype combinations of *ACE* (*I/D*) and *PAI-1* (*4G/5G*) is displayed in Table II. The frequency of the co-occurrence of the *II* genotype of *ACE* and *4G/4G PAI-1* was higher in controls than in the RM group (10.0 *vs.* 5.9%, p=0.17). There was no discrepancy between the observed and the expected values in both groups.

Discussion

Links between RAS, fibrinolytic balance, and placental vascularization have been proven by many researchers [13]. There are also several studies concerning the *ACE I/D* and *PAI-14G/5G* genotypes in women with RM, individually and combined with the conflicted results. Buchholz et al., studied 184 Caucasian women with a history of at least two unexplained pregnancy losses before 25 weeks of gestations, and 127, age- and race-matched controls with ≥1 successful pregnancy and negative history of pregnancy loss. Among women with RM, the prevalence of the *ACE DD* genotype was 32.1 *vs.* 23.6% in controls (p=0.11) and the *PAI-1 4G/4G* genotype was 39.1% as compared to 32.3% in controls (p=0.22). Coexistence of the *4G/4G* and *DD* hypofibrinolytic genotypes revealed a statistically significant difference between the investigated groups (13.6% in RM *vs*. 4.7% in controls, p=0.01). The Cohran-Armitrage trend test also confirmed a relationship between the *4G/4G* and *DD* genotype combination */4G* and *DD* (p=0.02). Buchholz et al., suggested the need for further research in this field and reflection whether or not double homozygous individuals should be treated with low-molecular-weight heparin [8]. Six years later, Goodman et al., conducted a similar case-control study to evaluate the association between RM and *ACE*, *PAI-1* variants and obtained contradictory results. That study involved 120 women with RM and 84 fertile controls. No significant differences between the groups were observed [8]. Recently, Kim et al., analyzed the allele and genotype frequencies of the *PAI-1 4G/5G* and *ACE I/D* polymorphisms in 227 Korean women with RM and 304 controls. Genotype and allele distributions of both polymorphisms did not

DOI: 10.17772/gp/62203

Table I. Distribution of genotypes and alleles of the studied polymorphisms in RM women and controls.

Genotypes	Study group RM (n=152)		Control group (n=180)		OR	95%CI	p^a	p^b
	Observed value n (%)	Expected value n (%)	Observed value n (%)	Expected value n (%)				
PAI-1 -675 4G/5G								
4G/4G	50 (32.9)	33.1	63 (35.0)	34.4	0.91	0.58-1.44	0.69	0.7866
4G/5G	75 (49.3)	48.9	85 (47.2)	48.5	1.09	0.71-1.68	0.70	
5G/5G	27 (17.8)	18.0	32 (17.8)	17.1	0.99	0.57-1.76	1.00	
Total	152 (100.0)	100.0	180 (100.0)	100.0				
Alleles								
4G	175 (57.6)	—	211 (58.6)	—	0.96	0.70-1.31	0.79	
5G	129 (42.4)	—	149 (41.4)	—	1.04	0.77-1.42	0.79	
Total	304 (100.0)	—	360 (100.0)	—				
ACE I/D								
II	32 (21.1)	22.4	44 (24.4)	22.8	0.82	0.49-1.38	0.46	0.9166
ID	80 (52.6)	49.9	84 (46.7)	49.9	1.27	0.82-1.96	0.28	
DD	40 (26.3)	27.7	52 (28.9)	27.3	0.88	0.54-1.43	0.60	
Total	152 (100.0)	100.0	180 (100.0)	100.0				
Alleles								
I	144 (47.4)	—	172 (47.8)	—	0.98	0,72-1,33	0,92	
D	160 (52.6)	—	188 (52.2)	—	1.02	0,75-1,38	0,92	
Total	304 (100.0)	—	360 (100.0)	—				

p^a Chi-square Pearson Test
p^b Cochran-Armitage trend test

Table II. Genotype coexistence of the analyzed polymorphisms.

			PAI-1			p^b	total
			4G/4G n (%)	4G/5G n (%)	5G/5G n (%)		
ACE	II	RM	9 (5.9)	15 (9.9)	8 (5.3)	*0.19*	32 (21.1)
		control	18 (10.0)	19 (10.6)	7 (3.9)		44 (24.4)
		p^a	*0.17*	*0.84*	*0.55*		
	ID	RM	28 (18.4)	41 (27.0)	11 (7.2)	*0.51*	80 (52.6)
		control	27 (15.0)	42 (23.3)	15 (8.3)		84 (46.7)
		p^a	*0.40*	*0.45*	*0.71*		
	DD	RM	13 (8.6)	19 (12.5)	8 (5.3)	*0.85*	40 (26.3)
		control	18 (10.0)	24 (13.3)	10 (5.6)		52 (28.9)
		p^a	*0.65*	*0.82*	*0.92*		
	p^b		*0.55*	*0.96*	*0.63*		
	total RM		50 (32.9)	75 (49.3)	27 (17.8)		152 (100.0)
	total control		63 (35.0)	85 (47.2)	32 (17.8)		180 (100.0)

p^a Chi-square Pearson Test
p^b Cochran-Armitage trend test

differ between the groups. These authors concluded that both polymorphisms, either alone or in combination, are not major determinants of RM occurrence in Korean women [14].

In a study from the Gaza Strip, including 100 women with a positive history of at least 3 consecutive abortions before 25 weeks of gestation and 100 controls, Al Sallout et al., found no significant associations between *ACE I/D, PAI-1 4G/5G* and the occurrence of first-trimester RM. In their study, all women from the control group were in the postmenopausal period to exclude any abortion which could have occurred after the investigation. The *ACE DD* genotype was present in 49% of the study group and 54% of controls (p=0.479), genotype *4G/4G PAI-1* occurred in both groups at the same 16% frequency [15].

The results of the performed meta- analyses are also ambiguous. Researches of Chen and Li both showed an association of the *PAI-1 4G/5G* polymorphism and recurrent

pregnancy loss [16, 17]. In a meta-analysis by Chen et al., 18 case-control studies were included (3684 cases and 2208 controls). No significant association between the studied *4G/5G* polymorphism and the risk of RM under the recessive model (OR=1.70) was found. In subgroup analysis, correlations were significant for Asians and Africans under a recessive genetic model (OR=2.12 and 4.48, respectively) but not for Americans, Europeans and Oceanians [16]. Li et al., performed a meta-analysis based on 22 case–control studies, with 4306 cases and 3076 controls. It revealed that the *PAI-1 4G/5G* polymorphism was significantly associated with an increased risk for RM (OR=1.89; *p*=0.0003). In a race subgroup analysis, this polymorphism was associated with an increased RM risk in Caucasians (18 studies, OR=2.23; *p*=0.0003), but not in Asians (4 studies, OR=1.47; *p*=0.18) [17].

In a meta-analysis of 11 case-control studies evaluating the association between the *ACE I/D* polymorphism and RM, involving a total of 3357 subjects, Wang et al., found a significant association between *ID* and *DD* genotypes as compared to *II* (*DD vs. II p*= 0.003 and *ID vs. II p*<0.001). In their meta-analysis, the *D* allele contributed to an elevated risk for RM [18].

Su et al., conducted a meta-analysis to investigate the association between *PAI-1* and *ACE* polymorphisms in women with idiopathic recurrent pregnancy loss. They included 11 association studies for the *PAI-1 4G/5G* polymorphism and 11 for the *ACE I/D* polymorphism in women with RM. Five of these studies investigated both polymorphisms together. These authors found a statistically significant correlation with the *ACE I/D* polymorphism in studies including >2 recurrent abortions (OR=1.29; 95% CI 1.02-1.62) [19].

In a case-control study of 157 Czech women with RM and 74 healthy fertile women, Subrt et al., revealed a statistically significant correlation between RM and *PAI-1 (-675) 4G/4G* genotype, but no correlation between this polymorphism and the presence of 8 antiphospholipid antibodies in serum of RM women. Although our RM group was similar in sample size to the study of Subrt et al., (152 and 157 women, respectively), they observed the *4G* allele frequency (61.5% *vs*. 57.6%) slightly more often. In the control group from this article, the observed genotype distribution deviated significantly from the expected one. It is probably due to the small sample size of the control group [20].

In our study, heterozygous *ACE ID* was present in 52.6% of women with RM as compared to 46.7% of controls (p=0.16). The *DD* genotype was observed in 26.3% of the RM subjects vs. 28.9% of controls (p=0.35). The frequency of the ACE deletion allele was similar in both analyzed groups (52.6 vs. 52.2%). Genotype and allele frequencies of the *ACE* gene polymorphism in our controls were similar to those found in other studies in Poland (II: 23-35%, ID: 42-57% and DD 18-26%) [21, 22, 23]. The obtained results revealed no correlation of the *-675 4G/5G* and *I/D ACE* gene polymorphisms with the risk of RM. The frequency of the co-occurrence of the *II* genotype of *ACE* and *4G/4G PAI-1* was higher in controls than in the RM group (10.0 *vs.* 5.9%, *p*=0.12). Subjects homozygous for the *I* allele have lower tissue and plasma ACE concentrations than *ID* and *DD* genotypes and less angiotensin II. Low angiotensin II level increases uteroplacental blood flow and, additionally, angiotensin II activates the *PAI-1* gene expression through AT1R, thus inhibiting human trophoblast invasion [24].

RAS and fibrinolysis systems are complex, dependent on many proteins and receptors, and both undergo significant changes in pregnancy. All of the components of RAS are expressed already at 5 weeks of gestation in human embryos [25]. ACE may change the fibrinolytic balance because angiotensin II increases the expression of *PAI-1*. Also, bradykinin undergoes degradation, which causes stimulation of the t-PA production [26]. During pregnancy, the PAI-1 level increases and the placenta starts producing PAI-2. PAI-1 values are 5-fold higher at 35 weeks (37.8 AU/mL) than at 12 weeks of gestation (7.4 AU/mL). Although its level decreases within 1 day postpartum, the PAI-2 levels remain elevated for over 11 days [27, 28].

Conclusions

The obtained results suggest no apparent association between the *ACE I/D*, *PAI-1 4G/5G* polymorphisms and increased RM susceptibility in the analyzed Polish population.

Both studied genes *ACE* and *PAI-1* are expressed in many tissues and organs in both, the mother and the fetus. Also, several polymorphic loci exist in these genes so further studies of other polymorphisms and other genes involved in the renin-angiotensin and fibrinolytic systems are needed. Despite the lack of connection between the investigated polymorphisms and the frequency of RM in the presented analysis, RAS and fibrinolytic systems seem to be very promising subjects for further research.

Oświadczenie autorów:

1. Grażyna Kurzawińska – autor koncepcji i założeń pracy, przygotowanie manuskryptu i piśmiennictwa – autor zgłaszający i odpowiedzialny za manuskrypt.
2. Magdalena Barlik – współautor tekstu pracy, współautor protokołu, korekta i aktualizacja literatury.
3. Krzysztof Drews - opracowanie założeń pracy, weryfikacja merytoryczna pracy.
4. Agata Różycka – wykonanie badań laboratoryjnych współautor protokołu.
5. Agnieszka Seremak-Mrozikiewicz – współautor tekstu pracy, korekta i aktualizacja literatury.
6. Marcin Ożarowski – współautor tekstu pracy, opracowanie statystyczne wyników.
7. Andrzej Klejewski – współautor tekstu pracy, współautor protokołu, korekta i aktualizacja literatury.
8. Bogusław Czerny – współautor tekstu pracy, korekta i aktualizacja literatury.
9. Hubert Wolski – opracowanie koncepcji i założeń badań, analiza i interpretacja wyników, ostateczna weryfikacja i akceptacja manuskryptu.

Źródło finansowania:

Badania statutowe Kliniki Perinatologii i Chorób Kobiecych UM w Poznaniu – nr: 502-01-02218344-0003344

Konflikt interesów:

Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

DOI: 10.17772/gp/62203

References

1. Battinelli EM, Marshall A, Connors JM. The role of thrombophilia in pregnancy. *Thrombosis*. 2013, 516420.
2. Thornton P, Douglas J. Coagulation in pregnancy. *Best Pract Res Clin Obstet Gynaecol.* 2010, 24, 339-352.
3. Barlik M, Wolski H, Drews K, [et al.]. The contribution of Hind III C>G PAI-1 gene polymorphism in etiology of recurrent miscarriages. *Ginekol Pol.* 2015, 86 (4), 274-279.
4. Williams PJ, Mistry HD, Innes BA, [et al.]. Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human. *Placenta.* 2010, 31, 448-55.
5. Pan N, Frome WL, Dart RA, [et al.]. Expression of the renin-angiotensin system in a human placental cell line. *Clin Med Res.* 2013, 11, 1-6.
6. Pringle KG, Tadros MA, Callister RJ, Lumbers ER. The expression and localization of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in trophoblast invasion and angiogenesis? *Placenta.* 2011, 32, 956–962.
7. Dawson SJ, Wiman B, Hamsten A, [at el.]. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. *J Biol Chem.* 1993, 268, 10739-45.
8. Buchholz T, Lohse P, Rogenhofer N, [et al.]. Polymorphisms in the ACE and PAI-1 genes are associated with recurrent spontaneous miscarriages. *Hum Reprod.* 2003, 18, 2473-7
9. Goodman C, Hur J, Goodman CS, [et al]. Are polymorphisms in the ACE and PAI-1 genes associated with recurrent spontaneous miscarriages? *Am J Reprod Immunol.* 2009, 62, 365-370.
10. Ueda S, Heeley RP, Lees KR, [et al.]. Mistyping of the human angiotensin-converting enzyme gene polymorphism: frequency, causes and possible methods to avoid errors in typing. *J Mol Endocrinol.* 1996, 17, 27-30.
11. Doggen CJM, Bertina RM, Manger Cats V, [et al.]. The 4G/5G polymorphism in the plasminogen activator inhibitor – 1 gene is not associated with myocardial infarction. *Thromb Haemost.* 1999, 82, 115-20.
12. Zhang L., Jiang Y. (2013). MCPerm: A Monte Carlo permutation method for multiple test correlation. R package version 1.1.4. http://CRAN.R-project.org/package=MCPerm
13. Irani RA, Xia Y. The functional role of the renin-angiotensin system in pregnancy and preeclampsia. *Placenta.* 2008, 29, 763-71.
14. Kim JJ, Choi YM, Lee SK, [et al.]. The PAI-1 4G/5G and ACE I/D polymorphisms and risk of recurrent pregnancy loss: a case–control study. *Am J Reprod Immunol.* 2014, 72, 571-76.
15. Al Sallout RJ, Sharif FA. Polymorphisms in NOS3, ACE and PAI-1 genes and risk of spontaneous recurrent miscarriage in the Gaza Strip. *Med Princ Pract.* 2010, 19, 99-104.
16. Chen H, Nie S, Lu M. Association between plasminogen activator inhibitor-1 gene polymorphisms and recurrent pregnancy loss: a systematic review and meta-analysis. *Am J Reprod Immunol.* 2015, 73, 292-300.
17. Li X, Liu Y, Zhang R, [et al.]. Meta-analysis of the association between plasminogen activator inhibitor-1 4G/5G polymorphism and recurrent pregnancy loss. *Med Sci Monit.* 2015, 21, 1051-6.
18. Wang Z, Wang P, Wang X, [et al.]. Significant association between angiotensin-converting enzyme gene insertion/deletion polymorphism and risk of recurrent miscarriage: a systematic review and meta-analysis. *Metabolism.* 2013, 62, 1227-1238.
19. Su MT, Lin SH, Chen YC, Kuo PL. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. *Thromb Haemost.* 2013, 109, 8-15.
20. Subrt I, Ulcova-Gallova Z, Cerna M, [et al.]. Recurrent pregnancy loss, plasminogen activator inhibitor-1 (-675) 4G/5G polymorphism and antiphospholipid antibodies in Czech women. *Am J Reprod Immunol.* 2013, 70, 54-58.
21. Żak I, Niemiec P, Sarecka B, [et al.]. Carrier-state of D allele in ACE gene insertion/deletion polymorphism is associated with coronary artery disease, in contrast to the C677>T transition in the MTHFR gene. *Acta Biochim Pol.* 2003, 50, 527-534.
22. Niedoszytko M, Ratajska M, Chełmińska M, [et al.]. The angiotensinogen AGT p.M235T gene polymorphism may be responsible for the development of severe anaphylactic reactions to insect venom allergens. *Int Arch Allergy Immunol.* 2010, 153, 166-172.
23. Słowik A, Borratynska A, Pera J, [et al.]. II genotype of the angiotensin-converting enzyme gene increases the risk for subarachnoid hemorrhage from ruptured aneurysm. *Stroke.* 2004, 35, 1594-7.
24. Pan N, Frome WL, Dart RA [et al.]. Expression of the renin-angiotensin system in a human placental cell line. *Clin Med Res.* 2013, 11, 1-6.
25. Ueki N, Takeda S, Koya D, Kanasaki K. The relevance of the Renin-Angiotensin system in the development of drugs to combat preeclampsia. *Int J Endocrinol.* 2015, 572713.
26. Kramkowski K, Leszczynska A, Buczko W. Pharmacological modulation of fibrinolytic response - In vivo and in vitro studies. *Pharmacol Rep.* 2015, 67, 695-703.
27. Bremme K, Ostlund E, Almqvist I, [et al.]. Enhanced thrombin generation and fibrinolytic activity in normal pregnancy and the puerperium. *Obstet Gynecol.* 1992, 80, 132-137.
28. Brenner B. Haemostatic changes in pregnancy. *Thromb Res.* 2004, 114, 409-44.