Streszczenie

W ostatnich latach wiele uwagi poświęcono wariantom genów endoteliny-1 (ET-1 – endothelin-1) oraz enzymu konwertującego enotelinę-1 (ECE-1 – endothelin-1 converting enzyme) i związanym z nimi zmianom w stężeniu ET-1. Jednocześnie wskazano, że procesy te mogą być włączone w etiologię stanu przedrzucawkowego.

Cel pracy: *Celem pracy było ocena związku polimorfizmów genu ET-1 (Lys198Asn) oraz ECE-1 (Thr341Ile) z ryzykiem rozwoju nadciśnienia ciążowego i stanu przedrzucawkowego.*

Materiał i metody: *Badaniem objęto 110 ciężarnych z nadciśnieniem w ciąży (w tym 69 z nadciśnieniem ciążowym oraz 41 ze stanem przedrzucawkowym). Grupę kontrolną stanowiło 150 zdrowych kobiet ciężarnych. Częstość występowania genotypów badanych polimorfizmów oznaczano przy użyciu metody reakcji łańcuchowej polimerazy oraz metody polimorfizmu długości fragmentów restrykcyjnych (PCR/RFLP, polymerase chain reaction/ restriction fragment length polymorphism).*

Wyniki: *W pracy nie zaobserwowano statystycznie istotnych różnic w częstości występowania ET-1 Lys198Asn oraz ECE-1 Thr341Ile polimorficznych wariantów pomiędzy ciężarnymi z nadciśnieniem a grupą kontrolną. Żadnych znaczących różnic nie odnotowano również pomiędzy grupami GH i PE w porównaniu do grupy kontrolnej. Jednak równoległa analiza obydwu Thr341Ile ECE-1 oraz Lys198Asn ET-1 wariantów pokazała wyższą częstość występowania heterozygotycznego genotypu ECE-1 CT/ET-1 GT w grupie kontrolnej (5,3%) niż w całej grupie badanej, jak również w grupach GH i PE (0,9%, 1,4% oraz 0,0% odpowiednio, p=ns). W grupie kobiet z PE skurczowe ciśnienie krwi było wyższe u nosicielek genotypu GG polimorfizmu Lys198Asn ET-1 (180,7mmHg) w porównaniu do pacjentek z obecnością co najmniej jednego zmutowanego allela T (GT oraz TT) (167,3mmHg, p=ns). Najniższe ciśnienie krwi było związane z obecnością zmutowanego genotypu TT polimorfizmu Lys198Asn ET-1.*

Wyniki: *Powyższe rezultaty wskazują na brak bezpośredniego związku polimorfizmu Lys198Asn genu ET-1 oraz Thr341Ile genu ECE-1 z ryzykiem rozwoju nadciśnienia ciążowego i stanu przedrzucawkowego w badanej populacji kobiet polskich. Wysoka częstość współwystępowania heterozygotycznych genotypów ECE-1 CT/ET-1 GT polimorfizmów Thr341Ile ECE-1 i Lys198Asn ET-1 w grupie kontrolnej w porównaniu do grupy GH i PE sugeruje protekcyjną rolę obydwu zmutowanych alleli w rozwoju PE. Nosicielstwo zmutowanego genotypu TT polimorfizmu Lys198Asn ET-1 prawdopodobnie związane jest z niższymi wartościami ciśnienia skurczowego u kobiet ze stanem przedrzucawkowym. Potwierdzenie roli analizowanych polimorfizmów w etiologii nadciśnienia ciążowego i stanu przedrzucawkowego wymaga dalszych badań.*

Słowa kluczowe: **stan przedrzucawkowy** / **nadciśnienie ciążowe** / **endotelina-1** / / **enzym konwertujący endotelinę-1** / **polimorfizm genetyczny** /

Introduction

In the last few years intense research on the mechanisms of hypertension susceptibility in pregnancy has been conducted. In the light of these studies, changes leading to the development of this disease are explained by endothelium damage of spiral arteries during trophoblast invasion to the vessel wall. At the beginning of normal pregnancy trophoblast changes the muscle layer of the spiral arteries to modulate low-resistant system which assures proper perfusion of the placenta. Several important factors, such as nitric oxide, vascular endothelial growth factor and endothelin-1, determine uncomplicated course of a pregnancy and optimal fetal growth by control of the proper angiogenesis, blood-flow regulation and evolution of utero-placental unit. In preeclampsia, normal trophoblast invasion is limited only to decidual part of spiral arteries leading to the placental anoxia, endothelial damage, connected with imbalance of vasodilatator and vasoconstrictor levels [1, 2, 3].

During this pathological process, an increase of tromboxan A2 and endothelin-1, as well as adecrease of nitric oxide concentrations in plasma, impair proper balance between vasoactive factors, especially disturbances in endothelin-1 secretion which start in the early stage of pregnancy, intensify during the second half of pregnancy and are manifested clinically by preeclampsia symptoms [4, 5, 6, 7].

Endothelin (ET) is a paracrine hormone (21 amino-acid peptide) and a potent vasoconstrictor synthesized mainly by endothelium of blood vessels. Three different isoforms of endothelin family (ET-1, ET-2 and ET-3) encoded by separate genes have been recognized [8, 9]. The best known is ET-1 which is involved in cell proliferation, acid-alkali balance, inflammation processes, hemostasis and cardiovascular system function. ET-1 is synthesized through multiple proteolytic steps in a process regulated by endothelin-1 converting enzyme (ECE-1) [8, 10], localized mainly in endothelial cells which are simultaneously the source of ET-1.

ECE-1 protein is an integral membrane part and its activity is probably regulated by vascular endothelial growth factor, interleukin-1 and tumor necrosis factor alpha which increase ET-1 and ECE-1 mRNA expression [11]. Thus, ECE-1 is recognized as an integral part of ET-1 and both elements are known as endothelin-1 system. Recently, three different isoforms of ECE-1 (ECE-1a, -1b and -1c) have been described (differing only in their N-terminal regions), from which the ECE-1a isoform is suggested to have regulatory function because of the presence of potential binding sites for numerous transcription factors [12, 13].

Both elements of endothelin-1 system, ET-1 and ECE-1, contribute to pathogenesis of numerous, mainly vascular,

diseases. Increased concentration of ET-1 protein is observed in arterial hypertension, heart infarction, acute kidney failure, acquired heart defects and diabetes mellitus [14, 15].

In addition, it was also indicated that ECE-1 mRNA and enzyme activity is enhanced in atherosclerotic lesions. Furthermore, ET-1 and ECE-1 seem to be very important agents of the development of preeclampsia. Any changes in ECE-1 activity and quantity may influence the proper evolution of feto-placental unit and the development of preeclampsia. Recent studies show that polymorphisms of ET-1 and ECE-1 genes may affect ET-1 system and suggest that conditioned by genetic polymorphism changes in this system could take part in the development of preeclampsia [8, 9, 16].

The aim of this study was to evaluate the correlation between polymorphisms of ET-1 (*Lys198Asn*) and ECE-1 (*Thr341Ile*) genes and the risk of gestational hypertension and preeclampsia.

Material and methods

The study group consisted of 110 hypertensive pregnant women (mean: age 29.4±4,5 years, gestational age 36.9±3,5 weeks, systolic blood pressure 167.8±16,9 mmHg and diastolic blood pressure 104.3±11.6 mmHg) and the study group was divided into two subgroups: 69 pregnant women with gestational hypertension (GH group) and 41 preeclamptic pregnant women (PE group). Gestational hypertension and preeclampsia were recognized according to ACOG (*American Congress of Obstetricians and Gynecologists*) guidelines. The control group included 150 healthy pregnant women (mean: age 28.3±4.4 years, gestational age 39.1±1.3 weeks, systolic blood pressure 112.1±10.7 mmHg and diastolic blood pressure 70.6±9.1 mmHg). (Table I).

Women with multiple pregnancy, diabetes, thrombophilic complications and vascular changes were excluded from the analysis.

Patients were enrolled into the study from January 2006 to April 2009, in Division of Perinatology and Women's Diseases, University of Medical Sciences, Poznan, Poland. All patients gave their informed written consent to participate in the project. The goals of the investigation were approved by Bioethical Committee of Poznan University of Medical Sciences.

The frequency of investigated polymorphisms was examined by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method. DNA was isolated from white blood cells with QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany).

For ET-1 *Lys198Asn* gene polymorphism, the forward primer, containing a mismatched nucleotide to incorporate a restriction site (underlined penultimate T), was F 5'-TCA TGA TCC CAA GCT GAA AGG CTA-3', the reverse primer was R-5`ACC TTT CTT GGA ATG TTT TGA AC-3' (TibMolBiol, Poland).

The conditions of PCR (Dyad DNA Engine thermocycler, Bio-Rad, USA) were as follows: initial denaturation 95°C for 4 min., next 30 cycles (30 s for 94°C, 30 s for 60°C, 60 s for 72°C). Final synthesis lasted 10 min. in temperature 72°C. The obtained product (228 bp) was hydrolyzed with *Nhe*I restriction enzyme. The fragments obtained in electrophoresis were as follows: *GG* 203, 25 bp, *GT* 228, 203, 25 bp, *TT* 228 bp.

For analysis of *Thr341Ile* ECE-1 gene polymorphism, two primers (TibMolBiol, Poland) were used: F 5`-TAG AGC CCT GGG CTG TGA GGA GGA GC-3` and R 5`-CTT ACC ATC TGT CGG TGG TGT TGA TG-3`.

Table I. Clinical characteristic of investigated pregnant women.

	Study group	***Control group***	***p***
Age (years)			
mean ± SD *median* *min/max*	29.4 ±4.5 29 18/38	28.3 ±4.4 28 17/41	ns
Gestational age at delivery (weeks)			
mean ± SD *median* *min/max*	36.9 ±3.5 38 27/42	39.1 ±1.3 39 37/42	<0.0001
Systolic blood pressure (mmHg)			
mean ± SD *median* *min/max*	167.8 ±16.9 170 140/220	112.1 ±10.7 110 85/140	<0.001
Diastolic blood pressure (mmHg)			
mean ± SD *median* *min/max*	104.3 ±11.6 100 70/140	70.6 ±9.1 70 50/95	<0.001

Table II. Frequency of genotypes and alleles of *Lys198Asn* ET-1 gene polymorphism in the group of hypertensive and healthy pregnant women.

ET-1 Lys198Asn	*Hypertensive pregnants (GH + PE) (n=110)*		*GH (n=69)*		*PE (n=41)*		*Control group (n=150)*	
Genotypes	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)
GG	74 (67.3)	66.2	47 (68.1)	67.0	27 (65.8)	64.8	102 (68.0)	67.2
GT	31(28.1)	30.3	19 (27.5)	29.7	12 (29.3)	31.4	42 (28.0)	29.5
TT	5 (4.6)	3.5	3 (4.4)	3.3	2 (4.9)	3,8	6 (4.0)	3.3
total	110 (100.0)	100.0	69 (100.0)	100.0	41 (100.0)	100,0	150 (100.0)	100.0
Alleles								
G	179 (81.4)	-	113 (81.9)	-	66 (80.5)	-	246 (82.0)	-
T	41(18.6)	-	25 (18.1)	-	16 (19.5)	-	54 (18.0)	-
total	220 (100.0)	-	138 (100.0)	-	82 (100.0)	-	300 (100.0)	-

Table III. Frequency of genotypes and alleles of *Thr341Ile* ECE-1 gene polymorphism in the group of hypertensive and healthy pregnant women.

ECE-1 Thr341Ile	*Hypertensive pregnants (GH + PE) (n=110)*		*GH (n=69)*		*PE (n=41)*		*Control group (n=150)*	
Genotypes	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)	observed value n (%)	expected value (%)
CC	101 (91.8)	91.9	63 (91.3)	91.5	38 (92.7)	92.8	134 (89.3)	89.6
CT	9 (8.2)	7.8	6 (8.7)	8.3	3 (7.3)	7.1	16 (10.7)	10.1
TT	0 (0.0)	0.3	0 (0.0)	0.2	0 (0.0)	0.1	0 (0.0)	0.3
total	110 (100.0)	100.0	69 (100.0)	100.0	41 (100.0)	100.0	150 (100.0)	100.0
Alleles								
C	211 (95.9)	-	132 (95.6)	-	79 (96.3)	-	284 (94.7)	-
T	9 (4.1)	-	6 (4.4)	-	3 (3.7)	-	16 (5.3)	-
total	220 (100.0)	-	138 (100.0)	-	82 (100.0)	-	300 (100.0)	-

Conditions of PCR (Dyad DNA Engine thermocycler, Bio-Rad, USA) for examined ECE-1 polymorphism were as follows: initial denaturation 95°C for 4 min, subsequently 30 cycles (95°C for 30 s, 64°C for 30 s, 72°C for 30 s), final synthesis 72°C for 10 min. The obtained PCR product (210 bp) was hydrolyzed by restriction enzyme (Fast Digest *Bst*YI, Fermentas, Lithuania) and submitted to electrophoresis. Obtained fragments were as follows: homozygote *CC* – 172, 38 bp, heterozygote *CT* – 172, 111, 61, 38 bp, homozygote *TT* – 111, 61, 38 bp. The results were documented with the UVI-KS4000i/Image PC System (Syngen Biotech, USA).

Statistical analysis was performed using the SPSS v. 17.0 software. *p* value of less than 0,05 was considered statistically significant.

Results

The frequency of particular genotypes of ET-1 *Lys198Asn* gene polymorphism in the whole study group was as follows: *GG : GT : TT* = 67.3 : 28,1 : 4.6% and in the control group: *GG : GT : TT* = 68.0 : 28,0 : 4.0%. There were no statistically significant differences between hypertensive pregnant women and the control group. There were also no remarkable differences between GH and PE groups when compared to the controls. The frequency of mutated *TT* genotype was 4.4% in GH group, 4,9% in PE group and 4.0% at controls (*p*=ns), the similar observation was linked to the frequency of mutated *T* allele (18.1 *vs.* 19.5 *vs.* 18.0% in GH, PE and controls, respectively, *p*=ns). (Table II).

According to the *Thr341Ile* polymorphism of ECE-1 gene, no significant differences between study and control groups

Table IV. Coexistence of both *Lys198Asn* ET-1 and *Thr341Ile* ECE-1 investigated polymorphisms.

			ET-1 Lys198Asn n (%)			*Total*
			GG	*GT*	*TT*	
Hypertensive pregnant (GH + PE) (n=110)	*ECE-1 Thr341Ile*	*CC*	66 (60.0)	30 (27.3)	5 (4.5)	101 (91.8)
		CT	8 (7.3)	1(0.9)	0 (0.0)	9 (8.2)
		TT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		total	73 (67.3)	32 (28.2)	5 (4.5)	110 (100.0)
GH (n=69)	*ECE-1 Thr341Ile*	*CC*	42 (60.9)	18 (26.1)	3 (4.3)	63 (91.3)
		CT	5 (7.3)	1 (1.4)	0 (0.0)	6 (8.7)
		TT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		total	47 (68.2)	19 (27.5)	3 (4.3)	69 (100.0)
PE (n=41)	*ECE-1 Thr341Ile*	*CC*	24 (58.5)	12 (29.3)	2 (4.9)	38 (92.7)
		CT	3 (7.3)	0 (0.0)	0 (0.0)	3 (7.3)
		TT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		total	27 (65.8)	12 (29.3)	2 (4.9)	41 (100.0)
Control group (n=150)	*ECE-1 Thr341Ile*	*CC*	94 (62.7)	34 (22.7)	6 (4.0)	134 (89.3)
		CT	8 (5.3)	8 (5.3)	0 (0.0)	16 (10.7)
		TT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		total	102 (68.0)	42 (28.0)	6 (4.0)	150 (100.0)

have been observed either. The frequency of homozygotic *CC* genotype in the study group was 91.8 *vs.* 89.3% in the control group (OR=1.34, 95%CL 0.53-3.58, *p*=ns) and frequency of heterozygotic *CT* genotype was 8.2 *vs.* 10.7%, respectively (OR=0.75, 95%CL 0.27-1.88, *p*=ns). Likewise, frequencies of wild type *C* allele (95,9 *vs.* 94.7%, OR=0.75, 95%CL 0.29-1.86, *p*=ns) and mutated *T* allele (4.1 *vs.* 5.3%, OR=1.32, 95%CL 0.53-3.54, *p*=ns) were comparable between controls and the study group. The frequencies of particular genotypes were similar in GH and PE groups. We have not observed any presence of mutated *TT* genotype in GH, PE and the control groups. (Table III).

Additionally, we have analyzed the ECE-1 and ET-1 variant localization of both *Thr341Ile* ECE-1 and *Lys198Asn* ET-1 investigated polymorphisms. The frequencies of *ECE-1 CC/ET-1 GG, ECE-1 CC/ET-1 TT*, and *ECE-1 CT/ET-1 GG* genotypes were similar in both groups. More frequent genotypes *ECE-1 CC/ET-1 GT* coexisted in the whole study group of hypertensive pregnant women (27.3%, *p*=ns), GH group (26.1%), and PE group (29.3%) than in the controls (22.7%, *p*=ns). The most interesting observation in our study was higher presence of both *ECE-1 CT/ET-1 GT* heterozygotic genotypes in the control group (5.3%) when compared to the whole study, GH and PE groups (0.9%, 1.4% and 0.0% respectively, *p*=ns).

Lack of *ECE-1 TT/ET-1 GG, ECE-1 TT/ET-1 GT, ECE-1 CT/ET-1 TT*, and *ECE-1 TT/ET-1 TT* joint genotypes in both analyzed groups has been observed. (Table IV).

The last step involved the analysis of the connection of blood pressure with particular genotypes of investigated polymorphisms. The most interesting observation was made in preeclamptic women: higher systolic blood pressure values were found in *GG Lys198Asn* ET-1 carriers (180.7mmHg) after comparing to carriers of genotypes containing at least one mutated *T* allele (*GT + TT*) (167,3mmHg, *p*=ns).

In connection to the *Thr341Ile* ECE-1 gene polymorphism, the systolic blood pressure was slight higher in *CC* genotype carriers than in carriers of mutated *T* allele (*CT + TT*) (for systolic: 177.2 *vs.* 170.0mmHg, *p*=ns).

Also, the analysis of genotypes coexistence showed clearly that in preeclamptic women the lowest blood pressure level was connected with the *TT* mutated genotypes of *Lys198Asn* ET-1 gene polymorphism presence. (Table V and VI).

Discussion

Endothelial dysfunction, remarkable in the second stage of preeclampsia, is clearly connected with endothelin-1 system disturbances. Thus, studies related to this problem cover the changes of ET-1 and ECE-1 activity.

Table V. Blood pressure level in the group of preeclamptic women in connection with genotypes of *Lys198Asn* ET-1 and *Thr341Ile* ECE-1 investigated polymorphisms.

		PE group (n=41)	
		Systolic blood pressure (mmHg)	***Diastolic blood pressure (mmHg)***
ET-1 Lys198Asn	***GG***		
	average	180.7±15.9	107.6±12.4
	median	180	110
	range	160-220	70-120
	GT		
	average	169.6±18.2	102.1±11.6
	median	167.5	100
	range	150-210	80-120
	TT		
	average	165.0±7.1	102.5±3.5
	median	165	102.5
	range	160-170	100-105
ECE-1 Thr341Ile	***CC***		
	average	177.2±17.4	105.9±12.1
	median	175	107.5
	range	150-220	70-120
	CT		
	average	170.0±10.0	103.3±11.6
	median	170	110
	range	160-180	90-110
	TT	-	-
	average		
	median		
	range		

In the study made by Słowiński *et al.,* there was significantly higher concentration of ET-1 in serum from preeclamptic women (30 subjects, 24-36 weeks gestation) compared to 125 healthy pregnancies. Similar results showing the important role of ET-1 changes in hypertensive women were obtained by other researchers [17, 18, 19]. Moreover, Margarit *et al.* noted increased ET-1 concentration in amniotic fluid in the second trimester of pregnancy in women who subsequently developed preeclampsia [20]. The study of Baksu *et al.* revealed that not only increased ET-1 concentration but also decreased concentration of nitric oxide and impaired balance of those two substances play an important pathogenetic role in the development of preeclampsia [21]. On the other hand, Ajne *et al.* analyzed ECE-1 activity and concentration in women with preeclampsia and showed higher ECE-1 synthesis in hypertensive patients compared to healthy pregnant women [22].

Considering multifactorial background of preeclampsia, especially including the genetic variants, it was hypothesized that many genetic polymorphisms are involved in its pathogenesis. Furthermore, the changes of ET-1 and ECE-1 concentration observed in preeclamptic women could be conditioned by different polymorphic variants of the genes coding for these elements. Aggarwal *et al.* analyzed the connection of *G5665T* gene ET-1 polymorphism with the risk of PE development and ET-1 concentration in serum (120 preeclamptic women and 118 healthy pregnant women). Additionally, placenta fragments were investigated for ET-1 activity (20 PE women and 24 healthy pregnant women). Higher level of ET-1 (1.45±0.55 *vs.* 0.91±0.42 pg/ml; $p<0.0001$) and more frequent *T* allele of *G5665T* gene ET-1 polymorphism (0.43 *vs.* 0.28; $p=0.04$) have been observed in serum of PE women. Furthermore, higher ET-1 concentration in women carrying at least one mutated *T* allele (1.08±0.48 vs. 1.31±0.59 pg/ml; $p=0.004$) has been noted. ET-1 activity in placenta from PE women was lower ($p<0.001$). In PE, the ET-1 released from maternal endothelium is increased, contrary to endothelium in placental vessels, where decreasing level of ET-1 is suggested to be one of the compensation forms of disturbances in utero-placental flow [23]. It was also suggested the possible interaction between other genetic polymorphisms from ET-1 system and PE development. However, association of *-231G>A* polymorphism of endothelin receptor gene with preeclampsia has not been confirmed [24].

Attempts have been made to correlate the presence of genetic polymorphisms with blood pressure levels in preeclamptic pregnant women. Barden *et al.* analyzed the connection between *Lys198Asn* ET-1 gene polymorphism and pathogenesis of preeclampsia. In the study group there were 72 preeclamptic women and 81 healthy pregnant controls. The authors did not show directly the involvement of this genetic variant in the development of PE. Interestingly, the occurrence of *T* allele

Table VI. Blood pressure level in the group of preeclamptic women in connection with both *Lys198Asn* ET-1 and *Thr341Ile* ECE-1 genotype polymorphisms coexistence.

			ET1 Lys198Asn n (%)		
			GG (mmHg)	***GT (mmHg)***	***TT (mmHg)***
Systolic blood pressure (mmHg) in PE group (n=41)	***ECE Thr341Ile***	***CC*** *average* *median* *range*	182.1±21.2 180 160/220	169.6±7.1 167.5 150/210	165.0±7.1 165 160/170
		CT *average* *median* *range*	170.0±14.1 170 160/180	-	-
		TT *average* *median* *range*	-	-	-
Diastolic blood pressure (mmHg) in PE group (n=41)	***ECE Thr341Ile***	***CC*** *average* *median* *range*	108.1±14.1 110 70/120	102.1±21.2 100 80/120	102.5±3.5 102.5 100/105
		CT *average* *median* *range*	103.3±14.1 110 90/110	-	-
		TT *average* *median* *range*	-	-	-

(GT and *TT* genotypes) correlated with higher diastolic blood pressure level (121 *vs.* 116 mmHg in *GG* homozygotes) and presence of *TT* genotype with higher concentration of ET-1 in serum (5.8 pg/ml *vs.* 3.1 pg/ml in heterozygote *GT vs.* 3.6 pg/ml in *GG* homozygote). It was suggested that *Lys198Asn* ET-1 polymorphism could play a predictive role in the development of PE [25]. Although no correlation of *Lys198Asn* ET-1 gene polymorphism frequency and analyzed hypertensive disorders has been noted in our study, similarly to Barden and co-workers, an interesting observation was made in connection with genotypes and blood pressure. Contrary to research made by Barden et al., in our study *TT* genotype was related to lower systolic blood pressure level (165.0 *vs.* 180.7 mmHg, *p*=ns).

Impairment of ECE-1 activity may be determined by its gene polymorphisms but there is no study connected with pathogenesis of preeclampsia. In recent years many studies were focused on *338C>A* genetic variant of ECE-1 gene and its connection with cardiovascular diseases. Funalot *et al.* investigated the influence of the ECE-1 *338C>A* polymorphism on blood pressure levels in a large group of 1189 hypertensive non-pregnant women. Female carriers of *AA* homozygous genotype had higher systolic, diastolic and mean blood pressure. The authors concluded that ECE-1 gene variants may modulate blood pressure in women [26]. These results seem to be most intriguing from obstetric point of view. Results of Wang *et al.* show that the *338C>A* ECE-1 variant might be associated with increased risk of coronary artery disease in Chinese population [27]. Moreover, a connection of *338C>A* ECE-1 polymorphism with late onset Alzheimer's disease and autosomal dominant polycystic kidney disease has been established [28, 29]. Data concerning other interesting *Thr341Ile* ECE-1 gene polymorphism have been published by Adamkova *et al.* in the juvenile hypertensive patients. This study concerned genetic determination of endothelial function and the size of heart sections in juvenile hypertensives. Hypertensive patients with the *Thr341Ile* had a larger left ventricle septum and back wall of the left ventricle than patients in the control group [30]. These signals show a correlation between genetic variants of ECE-1 and impairment of endothelial functions and allow to recognize the endothelin-1 system as the essential initiating point leading to the cardiovascular system lesions which could also be involved in pathomechanism of preeclampsia.

To the best of our knowledge, this study is the first to monitor the significance of *Thr341Ile* ECE-1 polymorphism in relation to preeclampsia in Polish pregnant women. Therefore, the entire analysis of the role of this polymorphism and its coexistence with other genetic variants and PE risk is a challenge. Our results suggest lack of direct correlation between *Thr341Ile* ECE-1 polymorphism and the risk of GH and PE. Moreover, no connection between the second investigated *Lys198Asn* ET-1 polymorphism and pathogenesis of GH and PE has been noted.

Nevertheless, results of our study may suggest a weak genotype interaction of both analyzed ET-1 and ECE-1 polymorphisms and the risk of preeclampsia. The most interesting fact is a higher prevalence of *CT/GT* heterozygote genotypes coexistence of both *Thr341Ile* ECE-1 and *Lys198Asn* ET-1 polymorphisms in controls than in GH and PE groups. This could suggest a protective role of both mutated alleles in the development of these conditions and justify further studies.

Although the weakness of this study is a small group of preeclamptic women and lack of measurement of ET-1 and ECE-1 activity, this article presents up-to-date problem concerning genetic markers involved in the development of PE. Our study is also a confirmation of an important role of several substances involved in angiogenesis and vascular tense regulation which occur to be very important factors in many obstetric complications.

Conclusions

1. Results of this study suggest lack of direct correlation of *Lys198Asn* ET-1 and *Thr341Ile* ECE-1 gene polymorphisms with the risk of gestational hypertension and preeclampsia in studied population of Polish women.
2. High prevalence of *ECE-1 CT/ET-1 GT* heterozygote genotypes of both *Thr341Ile* ECE-1 and *Lys198Asn* ET-1 polymorphisms in healthy pregnant subjects compared to GH and PE groups suggests a protective role of mutated alleles in the development of PE.
3. The carrier of mutated *TT* genotype of *Lys198Asn* ET-1 polymorphism is probably connected with lower systolic blood pressure level in preeclamptic women.
4. Further studies are needed to establish the role of analyzed polymorphisms in the etiology of gestational hypertension and preeclampsia.

References:

1. López-Jaramillo P, Casas J, Serrano N. Preeclampsia: from epidemiological observations to molecular mechanisms. *Braz J Med Biol Res.* 2001, 34, 1227-1235.
2. Kim Y, Park H, Lee H, [et al.]. Reduced L-arginine level and decreased placental eNOS activity in preeclampsia. *Placenta.* 2006, 27, 438-444.
3. Escudero C, Puebla C, Westermeier F, Sobrevia L. Potential cell signaling mechanisms involved in differential placental angiogenesis in mild and severe pre-eclampsia. *Curr Vasc Pharmacol.* 2009, 7, 475-485.
4. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod.* 2003, 69, 1-7.
5. Wulff C, Weigand M, Kreienberg R, Fraser H. Angiogenesis during primate placentation in health and disease. *Reproduction.* 2003, 126, 569-577.
6. Gilbert J, Ryan M, La Marca B, [et al.]. Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction. *Am J Physiol Heart Circ Physiol.* 2008, 294, 541-550.
7. Myatt L, Webster R. Vascular biology of preeclampsia. *J Thromb Haemost.* 2009, 7, 375-384.
8. Łapiński R. Rola endotelin w fizjologii i patologii nerek. *Adv Clin Exp Med.* 2003, 12, 81-86.
9. Irzmański R, Serwa-Stępień E, Barylski M, [i wsp.]. Zaburzenia równowagi śródbłonkowej w nadciśnieniu tętniczym. Rola peptydów natriuretycznych i endoteliny. *Kardiol Pol.* 2005, 63, 457-461.
10. Banno M, Hanada H, Kamide K, [et al.]. Association of genetic polymorphisms of endothelin-converting enzyme-1 gene with hypertension in a Japanese population and rare missense mutation in preproendothelin-1 in Japanese hypertensives. *Hypertens Res.* 2007, 30, 513-520.
11. Rossi G, Pitter G. Genetic variation in the endothelin system: do polymorphisms affect the therapeutic strategies? *Ann N Y Acad Sci.* 2006, 1069, 34-50.
12. Shao R, Yan W, Rockey D. Regulation of endothelin-1 synthesis by endothelin converting enzyme-1 during wound healing. *J Biol Chem.* 1999, 274, 3228-3234.
13. Zolk O, Quattek J, Sitzler G, [et al.]. Expression of endothelin-1, endothelin converting enzyme, and endothelin receptors in chronic heart failure. *Circulation.* 1999, 99, 2118-2123.
14. Bryl W, Pupek-Musialik D. Wpływ terapii ACE-inhibitorem na stężenie endoteliny i wybrane parametry metaboliczne u młodych osób z nadciśnieniem tętniczym. *Pol Merk Lek.* 2006, 122, 21, 174-175.
15. Roggensack A, Zhang Y, Davidge S. Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. *Hypertension.* 1999, 33, 83-89.
16. Mohaupt M. Molecular aspects of preeclampsia. Mol Aspects Med. 2007, 28, 169-191.
17. Słowiński T, Neumayer H, Stolze T, [et al.]. Endothelin system in normal and hypertensive pregnancy. *Clin Sci* (Lond). 2002, 103, 446-449.
18. Ariza A, Bobadilla N, Halhali A. Endothelin 1 and angiotensin II in preeclampsia. *Rev Invest Clin.* 2007, 59, 48-56.
19. LaMarca B, Alexander B, Gilbert J, [et al.]. Pathophysiology of hypertension in response to placental ischemia during pregnancy: a central role for endothelin? *Gend Med.* 2008, 5, 133-138.
20. Margarit L, Griffiths A, Tsapanos V, [et al.]. Second trimester amniotic fluid endothelin concentration. A possible predictor for pre-eclampsia. *J Obstet Gynaecol.* 2005, 25, 18-20.
21. Baksu B, Davas I, Baksu A, [et al.]. Plasma nitric oxide, endothelin-1 and urinary nitric oxide and cyclic guanosine monophosphate levels in hypertensive pregnant women. *Int J Gynaecol Obstet.* 2005, 90, 112-117.
22. Ajne G, Wolff K, Fyhrquist F [et al.]. Endothelin converting enzyme (ECE) activity in normal pregnancy and preeclampsia. *Hypertens Pregnancy.* 2003, 22, 215-224.
23. Aggarwal P, Jain V, Srinivasan R, [et al.]. Maternal EDN1 G5665T polymorphism influences circulating endothelin-1 levels and plays a role in determination of preeclampsia phenotype. *J Hypertens.* 2009, 27, 2044-2050.
24. Lisi V, Paternoster D, Stecca A, [et al.]. Investigation of endothelin-1 type A receptor gene polymorphism (-231 G>A) in preeclampsia susceptibility. *J Matern Fetal Neonatal Med.* 2007, 20, 145-149.
25. Barden A, Herbison C, Beilin L, [et al.]. Association between the endothelin-1 gene Lys198Asn polymorphism blood pressure and plasma endothelin-1 levels in normal and preeclamptic pregnancy. *J Hypertens.* 2001, 19, 1775-1782.
26. Funalot B, Courbon D, Brousseau T, [et al.]. Genes encoding endothelin-converting enzyme-1 and endothelin-1 interact to influence blood pressure in women: the EVA study. *J Hypertens.* 2004, 22, 739-743.
27. Wang L, Tang N, Zhu H, [et al.]. Endothelin-converting enzyme-1b C-338A polymorphism is associated with the increased risk of coronary artery disease in Chinese population. *Clin Chim Acta.* 2007, 384, 75-79.
28. Reiterová J, Merta M, Stekrová J, [et al.]. The influence of the endothelin-converting enzyme-1 gene polymorphism on the progression of autosomal dominant polycystic kidney disease. *Ren Fail.* 2006, 28, 21-24.
29. Scacchi R, Gambina G, Broggio E, [et al.]. C-338A polymorphism of the endothelin-converting enzyme (ECE-1) gene and the susceptibility to sporadic late-onset Alzheimer's disease and coronary artery disease. *Dis Markers.* 2008, 24, 175-179.
30. Adamkova V, Hubacek J, Pistulkova H, [et al.]. Genetic determination of an endothelial function and the size of the heart in juvenile hypertensives. *J Appl Biomed.* 2006, 4, 59-65.

Ginekol Pol. 2011, 82, 371-377

PRACE POGLĄDOWE
neonatologia

Czy operacje wewnątrzmaciczne są uzasadnione? – perspektywa neonatologa. Część I. Wrodzona Przepuklina Przeponowa

Are in-utero interventions justified? – perspective of neonatologists. Part I. Congenital Diaphragmatic Hernia (CDH)

Dąbrowska Katarzyna[1], Gadzinowski Janusz[1,2]

[1] Instytut Centrum Zdrowia Matki Polki, Klinika Neonatologii, Łódź, Polska
[2] Katedra i Klinika Neonatologii Uniwersytetu Medycznego w Poznaniu, Poznań, Polska

Streszczenie

Wstęp: *Operacje wewnątrzmaciczne płodu budzą wiele nadziei u rodziców. Ponieważ po porodzie to neonatolodzy zajmują się nie tylko chorym noworodkiem, ale także rodzicami, których optymizm często przekracza realia, postanowiliśmy spojrzeć na obecny stan wiedzy o tych zabiegach z punktu widzenia neonatologa. W pierwszej z trzech części dokonaliśmy analizy literatury dotyczącej zabiegów wewnątrzmacicznej korekcji wrodzonej przepukliny przeponowej (CDH).*

Cel: *Celem naszej pracy była ocena, czy dostępne w literaturze wyniki ponad 20 lat doświadczeń uzasadniają oferowanie i wykonywanie tych zabiegów.*

Metoda: *Dokonaliśmy przeglądu literatury na temat zabiegów wewnątrzmacicznych wykonywanych u płodów z CDH ze szczególnym uwzględnieniem badań randomizowanych z grupą kontrolną opublikowanych w Pubmed i bibliotece Cochrana.*

Wyniki: *W jedynym opublikowanym dotychczas badaniu randomizowanym z grupą kontrolną interwencja wewnątrzmaciczna nie poprawiła rokowania. Wyniki badań niekontrolowanych sugerują, że zabiegi te mogą poprawiać rokowanie w grupie pacjentów z ciężką formą CDH. Skuteczność późnej interwencji u pacjentów z umiarkowanie ciężką postacią jest obecnie oceniana w randomizowanych badaniach z grupą kontrolną.*

Wnioski: *Dzisiaj istnieją mało oczywiste dowody przemawiające za zastosowaniem interwencji wewnątrzmacicznych w ciąży powikłanej obecnością CDH u płodu, ale nie można wykluczyć, że dla ściśle określonej grupy pacjentów z CDH mogą oferować szansę poprawy rokowania. Kryteria kwalifikacji pacjentów czy optymalny wiek płodowy, w którym te zabiegi powinny być wykonywane nie są jasno sprecyzowane. Jakość życia i odległe powikłania związane z CDH, a nie tylko przeżywalność, powinny stać się podstawą oceny skuteczności tej terapii. Na chwilę obecną wydaje się, że zabiegi wewnątrzmaciczne powinny być przeprowadzane tylko w wyspecjalizowanych ośrodkach, w warunkach dobrze kontrolowanego eksperymentu medycznego.*

Słowa kluczowe: **wady wrodzone** / **przepuklina przeponowa** / **diagnostyka prenatalna** / / **chirurgia płodu** / **zabiegi wewnątrzmaciczne** /

Adres do korespondencji:
Janusz Gadzinowski
Katedra i Klinika Neonatologii Uniwersytetu Medycznego w Poznaniu
60 – 535 Poznań, ul. Polna 33, Polska
tel./fax.: (61) 659 9270
e–mail: jgadzin@gpsk.am.poznan.pl

Otrzymano: **16.06.2010**
Zaakceptowano do druku: **29.04.2011**