The importance of rs1021737 and rs482843 polymorphisms of cystathionine gamma-lyase in the etiology of preeclampsia in the Caucasian population

Znaczenie polimorfizmów rs1021737 i rs482843 gamma-liazy cystationinowej w etiologii stanu przedrzucawkowego w populacji kaukaskiej

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Abstract

Introduction: *Recently, an increasing number of reports indicate the participation of genetic factors in the pathogenesis of preeclampsia (PE). The genes involved in the synthesis of nitric oxide that participates in the vasolidation, may play an important role in the development of this disorder. Hydrogen sulfide (H2S) which is produced by cystathionine gamma-lyase exhibits a similar effect to nitric oxide. It is suggested that certain polymorphisms of the CTH gene may participate in the development of chronic hypertension and preeclampsia.*

Aim of the study: *To evaluate the frequency of genotypes and alleles of rs1021737 and rs482843 polymorphisms of CTH gene in women with preeclampsia from Wielkopolska region.*

Material and methods: *The study group consisted of 60 patients with diagnosed preeclampsia, into the control group 120 healthy pregnant women were enrolled. The examined rs1021737 and rs482843 polymorphisms of CTH gene were determined using PCR-RFLP method.*

Results: *Analysis of rs482843 polymorphism in the CTH gene showed a statistically significant difference in the prevalence of mutated GG genotype ($p<0.000001$) and mutated G allele ($p<0.000001$) in the group of pregnant women with PE compared to the control group. There was no such correlation for the rs1021737 polymorphism. Furthermore, there are also no relationship between studied polymorphisms and selected clinical and biochemical parameters.*

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Conclusions: *The results of rs482843 polymorphism analysis suggest that mutated GG genotype predisposes to preeclampsia occurrence. There was no such relationship for the rs1021737 polymorphism of CTH gene. Hence, further studies based on the determination of CSE expression level in women with PE may confirm the observed relationship between the rs482843 polymorphism and the risk of preeclampsia.*

Słowa kluczowe: **preeclampsia** / **hydrogen sulfide** / **cystathionine gamma-lyase** / / **genetic polymorphism** /

Streszczenie

Wstęp: *W ostatnim czasie coraz więcej doniesień wskazuje na udział czynników genetycznych w etiopatogenezie stanu przedrzucawkowego (PE – preeclampsia). Istotną rolę mogą odgrywać geny uczestniczące w syntezie tlenku azotu, głównego wazodylatora naczyń. Podobne działanie wazodyletacyjne pełni także siarkowodór (H2S) syntetyzowany przy udziale gamma-liazy cystationinowej. Sugeruje się, że określone polimorfizmy genu CTH mogą mieć znaczenie w rozwoju nadciśnienia przewlekłego i stanu przedrzucawkowego.*

Cel pracy: *Określenie częstości występowania genotypów i alleli polimorfizmów rs1021737 i rs482843 genu CTH w grupie kobiet ze stanem przedrzucawkowym z regionu Wielkopolski.*

Materiał i metody: *Grupa badana obejmowała 60 pacjentek ze zdiagnozowanym stanem przedrzucawkowym, natomiast grupa kontrolna stanowiła 120 zdrowych kobiet ciężarnych. Analizowane polimorfizmy rs1021737 i rs482843 genu CTH oznaczono przy pomocy metody PCR-RFLP.*

Wyniki: *Analiza polimorfizmu rs482843 genu CTH wykazała różnicę istotną statystycznie w częstości występowania genotypu zmutowanego GG (p<0.000001) oraz zmutowanego allela G (p<0.000001) w grupie kobiet ciężarnych z PE w stosunku do grupy kontrolnej. Nie zaobserwowano takiej zależności dla polimorfizmu rs1021737. Stwierdzono również brak zależności między badanymi polimorfizmami a wybranymi parametrami klinicznymi i biochemicznymi.*

Wnioski: *Uzyskane wyniki dotyczące polimorfizmu rs482843 genu CTH sugerują predyspozycję u ciężarnych nosicielek genotypu zmutowanego GG do wystąpienia stanu przedrzucawkowego. Nie wykazano takich uwarunkowań etiopatologicznych w odniesieniu do polimorfizmu rs1021737 genu CTH. Dalsze badania określające poziom ekspresji CSE u kobiet z PE mogą potwierdzić obserwowaną zależność pomiędzy polimorfizmem rs482843 a ryzykiem wystąpienia stanu przedrzucawkowego.*

Słowa kluczowe: **stan przedrzucawkowy** / **siarkowodór** / **gamma-liaza cystationinowa** / / **polimorfizm genetyczny** /

Introduction

Preeclampsia (PE) is the main cause of increased maternal and fetal morbidity and mortality. PE is a multifactorial condition, with strong evidence of genetic factors in disease causation. Numerous studies have searched for genes primarily responsible for its development.

The role of the following candidate genes in the etiology of PE has been emphasized: genes encoding for endothelin 1, endothelial nitric oxide synthase, coagulation factors II and V, genes responsible for the activity of the rennin-angiotensin system, and those responsible for drug transport [1, 2, 3].

In recent years, it has been claimed that the polymorphisms of genes encoding for vasodilator factors play an important role in the pathogenesis of preeclampsia. Hydrogen sulfide (H_2S), next to nitrogen oxide and carbon monoxide, is an important transmitter in the cardiovascular system. H_2S, synthesized by cystathionine gamma-lyase (CSE), could be a potent vasodilator of the placental vasculature. This compound also exhibits antioxidant effects and stimulates angiogenesis. Additionally, H_2S plays an important role in regulating the balance between growth and death of cells by inhibition of the CSE/H_2S pathway in the excessive apoptosis of vascular smooth muscle cells [4].

In 2008, Yang et al., pointed to the direct vasodilator effect of H_2S in the regulation of blood pressure [5]. Thus, abnormal function of the CSE/H_2S pathway is associated with the pathomechanism of cardiovascular diseases, including atherosclerosis and hypertension [6,7,8].

Endogenous H_2S is synthetized from α-cysteine either by cystathionine beta-synthase (CBS) (E.C.4.4.1.8) or cystathionine gamma-lyase (E.C.4.4.1.1) using pyridoxal 5'-phosphate (vitamin B_6) as a cofactor. Both enzymes belong to the family of lyases and are involved in several metabolic pathways, particularly in the transsulfuration process participating in the metabolism of methionine, homocysteine and cysteine [9,10]. Cystathionine beta-synthase catalyzes the formation of cystathionine from homocysteine and serine, while CSE participates in the conversion of cystathionine to alpha-ketobutyrate and cysteine [9,11]. Moreover, CBS can catalyze the formation of cystathionine and H_2S by condensation of cysteine and homocysteine, while CSE may participate in the H_2S synthesis from cysteine, with simultaneous formation of ammonia, thiocysteine and pyruvate [12, 13]. Thiocysteine decomposes to cysteine and H_2S [12]. Otherwise, H_2S can also be formed from mercaptopyruvate catalyzed by 3-mercaptopyruvate sulfurtransferase [11].

Cystathionine gamma-lyase is encoded by the *CTH* gene, which is located on chromosome 1 (1p31.1) [14]. Several *CTH* gene polymorphisms were identified in patients with cystathioninuria. Wang and Hegele indicated two nonsense mutations in exon 8 (*940-941delCT*) and exon 11 (*1220delC*), as well as two missense mutations, in exon 2 (*356C>T, T67I*) and exon 7 (*874C>G, Q240E*) [15]. On the other hand, these authors suggested the existence of a connection between functional polymorphism in exon 12 of the *CTH* gene (*1364G>T, rs1021737*) and the development of hypertension because this genetic variant converts serine at position 403 to isoleucine and may cause elevated plasma homocysteine levels. Therefore, it is suggested that genetic polymorphism in the gene encoding for CSE may correlate with severity of preeclampsia and influence high blood pressure values [15,16].

The aim of the study was to evaluate the frequency of genotypes and alleles of the *rs1021737* and *rs482843* polymorphisms of the *CTH* gene in preeclamptic and healthy pregnant women from the Polish population.

Material and methods

Patients

The patients were Caucasian, of Polish origin, recruited at the Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences. All participants gave their written informed consent. Local Ethics Committee approved of the study.

A total of 60 pregnant preeclamptic women (age 29.45±5.12 years, systolic blood pressure 160.92±20.22 mmHg, diastolic blood pressure 102.75±12.80 mmHg, gestational age at delivery 33.57±3.82 weeks) were enrolled in the study. PE was recognized according to the 2011 ACOG (*American College of Obstetricians and Gynecologists)* criteria [17]. Blood pressure was measured with the sphygmomanometer (twice, with a 6-hour interval) in a sitting or lying position in each woman. Urine was analyzed and proteinuria was recognized by the presence of ≥30 mg/dl of protein in a sample (or in test strip, with the 1+ result).

The control group included 120 healthy pregnant women (age 28.74±4.75 years, systolic blood pressure 108.50±11.59 mmHg, diastolic blood pressure 67.63±9.12 mmHg, gestational age at delivery 39.09±1.26 weeks). The exclusion criteria from the study were as follows: diabetes mellitus, obesity, cardiovascular diseases, chronic hypertension, renal and endocrinal diseases, thromboembolism, age <18 years, and multifetal pregnancy.

Genetic analysis

The molecular analysis was performed in the Laboratory of Experimental Pharmacogenetics, Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznan, Poland.

The frequency of the two investigated polymorphisms (*rs1021737* and *rs482843*) in the *CTH* gene was examined by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method. Primers for the *rs482843* polymorphism (*FORWARD* 5'-AGC AAC CCC GTT AGT TCC TT-3', *REVERSE* 5'-AGC TCT TGA CTT TCG CTT ATA AGC T-3'. PCR product - 197 bp) and rs1021737 polymorphism (*FORWARD* 5'-AGG GCA ATC ATG ACT CAT GCA TC-3', *REVERSE* 5'-TTG CAA AGG CTC ATT GTT GGT CC-3'. PCR product - 528 bp) used in the PCR reaction were described by Li et al. [18]. The obtained fragments after hydrolysis by restriction process are presented in Table I. Products of the electrophoresis were evaluated by visualization in the UV light (KS 4000/ Image PC of the Syngen Biotech Molecular Biology Instruments company).

Statistical analysis

SPSS 17.0 PL software was used for statistical analysis. The prevalence of certain genotypes was compared with the expected values using the chi-square test. Clinical and biochemical parameters and their relationship with the previously reported polymorphisms was assessed using one-way ANOVA test (SPSS Inc.). The values of $p<0.05$ were considered as statistically significant.

Results

Our study analyzed selected clinical and biochemical parameters in preeclamptic women and controls. Shorter gestational age at delivery (33.57±3.82 *vs*. 39.09±1.26 weeks, $p<0.0001$), as well as higher systolic (160.92±20.22 *vs*. 108.50±11.59 mmHg, $p<0.0001$) and diastolic (102.75±12.80 *vs*. 67.63±9.12 mmHg, $p<0.0001$) blood pressure were observed in PE subjects. These women were characterized by higher body weight both, before ($p<0.009$) and during pregnancy ($p<0.026$), as compared to controls. The study group also had higher values of hemoglobin ($p<0.0001$), erythrocytes ($p<0.0001$) and platelets (*ns*). In contrast to healthy women, protein was found in urine samples from the study group (16.8-1336.8 mg/dL, $p<0.0001$). A significant difference with respect to infant birth weight was observed between the PE group and controls (1963±887.49 *vs*. 3454.43±403.12 g, $p<0.0001$) (Table II).

Table I. Restriction enzymes and hydrolysis conditions.

Polymorphism	*Restriction enzyme*	*Recognized sequence*	*Time/temperature incubation*	*Length of fragments (bp)*
rs482843	*HindIII*	5'…A ↓ AGCTT…3' 3'…TTCGA ↑ A…5'	16 h, 37ºC	AA (172, 25) AG (197, 171, 25) GG (197)
rs1021737	*EcoRI*	5'…G ↓ AATTC…3' 3'…CTTAA ↑ G…5'	16 h, 37ºC	GG (528) GT (528, 429, 99) TT (429, 99)

Table II. Comparison of selected clinical parameters in preeclampsia and control groups.

Parameter	*Study group (PE) n=60*	*Control group n=120*	*p*
Age (years)			
mean±SD	29.45±5.12	28.74±4.75	*ns*
range	18-40	18-42	
median	30	29	
Gestational age at delivery (weeks)			
mean±SD	33.57±3.82	39.09±1.26	*p*<0.0001
range	25-40	37-42	
median	33	39	
Systolic blood pressure (mmHg)			
mean±SD	160.92±20.22	108.50±11.59	*p*<0.0001
range	110–220	90–140	
median	160	110	
Diastolic blood pressure (mmHg)			
mean±SD	102.75±12.80	67.63±9.12	*p*<0.0001
range	70–130	50–90	
median	100	70	
Height (cm)			
mean±SD	164.93±6.43	167.38±4.98	*ns*
range	149-176	150–180	
median	165	168	
Body weight before pregnancy (kg)			
mean±SD	64.56±15.64	60.33±9.38	*p*<0.009
range	42-128	40–97	
median	60	59.5	
Body weight during pregnancy (kg)	80.59±15.83	75.34±10.52	
mean±SD	55-139	52–106	*p*<0.026
range	76	59.5	
median			
Infant birth weight (g)			
mean±SD	1963±887.49	3454.43±403.12	*p*<0.0001
range	620-3940	2560-4640	
median	1850	3430	
Number of pregnancies			
mean±SD	1.53±0.83	1.55±0.79	*ns*
range	1–4	1–6	
median	1	1	
Delivery mode (n)			
Spontaneous birth	7 (11.67%)	56 (46,67%)	*p*<0.0001
Cesarean section	53 (88.33%)	64 (53.33%)	
Hemoglobin (mmol/l)			
mean±SD	8.46±2.54	6.72±0.79	*p*<0.0001
range	0.90–15.30	4.5–8.8	
median	7.80	6.775	
Erythrocytes (10^6/µl)			
mean±SD	4.28±0.95	3.68±0.45	*p*<0.0001
range	3.24–9.92	2.5–4.81	
median	4.08	3.685	
Leukocytes (10^3/µl)			
mean±SD	10.85±4.07	23.39±72.20	*ns*
range	4.14–25.29	8.27–806	
median	9.90	16.23	
Platelets (g/l)			
mean±SD	207.95±66.69	199.77±62.84	*ns*
range	88.00–398.00	9.1–314.6	
median	210.50	206.45	
Hematocrit (l/l)			
mean±SD	0.36±0.04	0.33±3.26	*ns*
range	0.20–0.45	0.219–0.36	
median	0.36	0.32	
Protein in urine (mg/dl)			
mean±SD	357.74±253.11	10.82±12.44	*p*<0.0001
range	166.8–1336.8	0-25	
median	500	0	

Table III. Frequency of genotypes of rs1021737 polymorphism of the CTH gene in women with preeclampsia and the control group.

	Study group (PE)		Control group	
	Observed n (%)	Expected %	Observed n (%)	Expected %
GG	27 (45.00)	46.69	52 (43.33)	45.00
GT	28 (46.67)	43.28	57 (47.50)	44.16
TT	5 (8.33)	10.03	11 (9.17)	10.84
Total	**60 (100.00)**	**100.00**	**120 (100.00)**	**100.00**
G	161 (67.08)		82 (68.33)	
T	79 (32.92)		38 (31.67)	
Total	**240 (100.00)**		**120 (100.00)**	

Table IV. Comparison of the odds ratio (OR) and 95% confidence interval (CI) for each genotype of the rs1021737 polymorphism.

Polymorphism rs1021737	OR	95% CI	p
GG	1.07	0.55 – 2.01	0.48
GT	0.97	0.49 – 1.88	0.52
TT	0.90	0.23 – 2.98	0.55
G	1.06	0.65 – 1.75	0.45
T	0.94	0.57 – 1.55	0.45

Table V. Frequency of genotypes of rs482843 polymorphism of the CTH gene in women with preeclampsia and the control group.

	Study group (PE)		Control group	
	Observed n (%)	Expected %	Observed n (%)	Expected %
AA	16 (26.67)	19.51	66 (55.00)	57.50
AG	21 (35.00)	49.32	50 (41.67)	36.66
GG	23 (38.33)	31.17	4 (3.33)	5.84
Total	**60 (100.0)**	**100.00**	**120 (100.00)**	**100.00**
A	53 (44.17)		182 (75.83)	
G	67 (55.83)		58 (24.17)	
Total	**120 (100.00)**		**240 (100.00)**	

Table VI. Comparison of the odds ratio (OR) and 95% confidence (CI) interval for each genotype of the rs482843 polymorphism.

Polymorphism rs482843	OR	95% CI	p
AA	0.29	0.14 – 0.61	0.00025
AG	0.75	0.37 – 1.49	0.24
GG	18.03	5.56 – 74.89	<0.000001
A	0.25	0.15 – 0.41	<0.000001
G	3.97	2.42 – 6.49	<0.000001

Analysis of the *rs1021737* polymorphism of the *CTH* gene revealed no statistically significant differences in the study group in relation to the frequency of genotypes (*GG* homozygote p=0.48, *GT* heterozygote p=0.52, mutated homozygous *TT* genotype p=0.55). In addition, a similar frequency of the *G* allele and the mutated *T* allele for the *rs1021737* polymorphism was observed in women with PE and controls (Table III and IV).

Individual genotypes for the *rs482843* polymorphism of the *CTH* gene in women with PE and the control group are presented in Table V. We showed an increased frequency of the mutated *GG* genotype in the study group as compared to controls (38.33% *vs*. 3.33%. p<0.000001). The occurrence of the mutated *GG* genotype for this polymorphism may predispose to PE, as evidenced by high values of the relative risk (OR=18.03) (Table VI).

We also observed higher frequency of the mutated *G* allele in women with PE as compared to controls (55.83% *vs*. 24.17%, $p<0.000001$). The wild *A* allele of the *rs482843* polymorphism of the *CTH* gene was observed in 75.83% of the healthy women, while in the study group its frequency was significantly lower and reached the value of 44.18% ($p<0.000001$). Analyzing the odds ratio for the examined alleles, we found that it is higher for the mutated *G* allele (OR=3.97) and may be associated with increased likelihood of developing PE during pregnancy (Table VI).

Moreover, comparing the biochemical parameters depending on the genotypes of the examined polymorphisms, there were no statistically significant differences between various subgroups of patients.

Discussion

Cystathionine gamma-lyase is the key enzyme responsible for H_2S synthesis in the cardiovascular system. Based on numerous studies, many CSE biological properties have been shown, including vasodilation, reduction of blood pressure, activation of NMDA receptors in the brain, decrease of CRH secretion from the hypothalamus, inhibition of myocardial contractility and vascular smooth muscle cell proliferation [5,19,20,21]. Moreover, it was concluded that H_2S deficiency, being an important product of CSE, may accelerate the development of atherosclerosis, especially in patients with hyperhomocysteinemia. Additionally, analyzing the effect of CSE inhibitors (e.g. propargylglycine), a decrease in plasma H_2S concentration by reduction of CSE synthesis in the aorta wall and an increase of blood pressure were observed [22]. Therefore, many authors pointed to the possibility of molecular diagnostics of cystathioninuria, which allows to carry out the studies of genotype-phenotype relationship and correlation of biochemical parameters, homocysteine level, and clinical implications, e.g. for vascular diseases [15,23,24]. Furthermore, the CSE enzyme deficiency may also be associated with the presence of polymorphic variants in the *CTH* gene. Therefore, experimental research focused on the analysis of the human *CTH* gene encoding for the CSE enzyme may be essential in elucidating the pathogenesis of chronic hypertension [15, 25, 26].

Wang et al., investigated the *rs1021737* (*c.1364G> T, S403I*) polymorphism in exon 12 of the *CTH* gene and total serum homocysteine levels in a group of 496 Caucasian. These authors indicated that total plasma homocysteine level could be an independent risk factor for atherosclerosis. Additionally, they showed that patients with mutated *1364TT* homozygous genotype had a significantly higher homocysteine levels as compared to carriers of other genotypes [25]. Moreover, it was found that the decrease of CSE activity is associated with the occurrence of cystathioninuria, as well as a decrease of H_2S synthesis [10]. Furthermore, Wang and Hegele hypothesized that reduced CSE activity influences the development of hereditary cystathioninuria [15].

Additionally, Kraus et al., investigated most of the novel polymorphisms of the *CTH* gene and CSE activity of the altered protein among the cystathioninemic/cystathioninuric patients. They observed that altered protein connected with mutated genotype is mainly characterized by reduced catalytic activity as compared to the wild genotype [27].

In another study, the possible influence of the *CTH* gene polymorphisms on the development of hypertension in Northern Chinese Han population has been demonstrated. The study was conducted in 503 hypertensive patients and 490 normotensive controls. However, the analyzed *rs482843* and *rs1021737* polymorphisms did show any impact on the development of hypertension among the study population [18].

In addition, Zhu et al., investigated how the presence of polymorphic variants in human *CTH* gene influences the kinetic properties of the CSE enzyme. These authors demonstrated that the *rs1021737 c.1364G>T* (*S403I*) polymorphism does not influence the CSE cofactor activity or steady-state kinetic properties of the enzyme. They also showed that the mutated *I403* variant has different frequency of alleles for each ethnic group, while the recessive variant is coupled with elevated plasma homocysteine levels [28].

So far, there have only been a few analyses searching for genetic *CTH* variants associated with the pathogenesis of preeclampsia [29,30,31]. The anti-angiogenic state in preeclampsia is generally confirmed. The H_2S with pro-angiogenic and anti-oxidative activity could play an important role in the pathogenesis of PE. On the other hand, few studies determined the role of H_2S in the modulation of feto-placental vasculature based on CSE expression in the placenta. The CSE presence was confirmed in smooth muscle cells of stem villi arteries. Moreover, studies concerning the expression of enzymes involved in the H_2S synthesis in the placenta are also very interesting. Both, the CBS and CSE enzymes are expressed in the placental endothelium. Additionally, the CBS enzyme is expressed in Hofbauer cells. It could be suggested that decreased CSE activity may be the reason of angiogenic imbalance, abnormal placentation, and may induce maternal hypertension.

Studies performed in the Chinese population indicated that H_2S plasma levels were significantly reduced in women with preeclampsia ($p<0.01$), which was simultaneously associated with reduced placental expression of CSE [29]. In another study, CSE activity was decreased in placentas from severe early-onset growth-restriction and preeclamptic subjects. These authors hypothesized that expression of CSE is reduced in placentas from preeclamptic and hypotrophic pregnancies with failure of placental vasculature [30].

Interesting results were obtained in a study by Holwerda et al., who indicated that CBS mRNA expression is statistically significantly reduced in early-onset preeclampsia ($p=0.002$), while mRNA expression of the CSE enzyme involved in H_2S synthesis remains unchanged [31].

To the best of our knowledge, our study has been the first to investigate the possible influence of the *rs482843* and *rs1021737* polymorphisms of the *CTH* gene on the development of PE in the population of Polish pregnant women. No correlation between genotypes and alleles of the *rs1021737* polymorphism and PE was observed. Analyzing the *rs482843* polymorphism, a higher frequency of the mutated *GG* genotype in women with PE as compared to the control group ($p<0.000001$, OR=18.027) was noted. These results suggest that the *rs482843* polymorphism of the *CTH* gene predisposes to the occurrence of PE in Polish pregnant women.

Obviously, further clinical studies concerning the polymorphic changes as the predisposing factors in the development of

vascular disease are needed. Therefore, it is also necessary to conduct further research on the assessment of CSE expression and protein level, as well as determination of H_2S plasma levels. These studies can provide important information about PE pathogenesis and find a suitable genetic marker that allows for early detection of women at risk for the development of PE.

Conclusions

Our results suggest a predisposition to preeclampsia in pregnant carriers of mutated variants of the *rs482843* polymorphism. There were no such correlations in relation to the *rs1021737* polymorphism of the *CTH* gene. Further studies based on the determination of CSE expression level in preeclamptic women could confirm the observed relationship between the *rs482843* polymorphism and the risk for preeclampsia.

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