ORIGINAL ARTICLE

UDC: 575:577.2]:618.3-06
https://doi.org/10.2298/VSP160329313K

Association between Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G Polymorphisms and Risk of Early-Onset Preeclampsia and Its Complications

Povezanost genskog polimorfizma Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T i IL-10 -1082 A>G sa rizikom od pojave rane preeklampsije i njenih komplikacija

Tijana Krnjeta*, Ljiljana Mirković[†‡], Svetlana Ignjatović[§‖], Dragana Tomašević[¶], Jelena Lukić[¶], Drina Topalov[¶], Nada Majkić-Singh**

***Roche d.o.o. Serbia BU Diagnostics, Belgrade, Serbia; Clinical Center of Serbia, [†]Clinic of Gynecology and Obstetrics, [§]Center for Medical Biochemistry, Belgrade, Serbia; University of Belgrade, [‡]Faculty of Medicine, Faculty of Pharmacy, [‖]Department of Medical Biochemistry, Belgrade, Serbia; [¶]Laboratory for Biochemistry and Molecular Diagnostics "Konzilijum", Belgrade, Serbia; **Society of Medical Biochemistry of Serbia, Belgrade, Serbia**

Abstract

Background/Aim. Preeclampsia (PE) belongs to the group of hypertensive disorders in pregnancy with the global average incidence of 2.16%. It is considered as one of the leading causes of maternal and neonatal morbidity and mortality worldwide. The goal of this study was to assess the potential association between Val158Met catechol-o-methyltransferase (COMT), tumor necrosis factor-alpha (TNF-α) -857 C>T, tumor necrosis factor receptor 1 (TNFR1) 36 A>G, interleukin-1alpha (IL-1α) 4845 G>T and interleukin-10 (IL-10) -1082 A>G polymorphisms and risk of early-onset preeclampsia (PE) and its complications. **Methods.** The study included 47 early-onset PE patients, which were grouped by disease severity and by size for gestational age and 47 control cases. The Val158Met polymorphism was genotyped by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis and inflammatory cytokine polymorphisms by the Sanger sequencing method. **Results.** The COMT Met allele as well as IL-1α T showed a protective role, decreasing the risk of early-onset PE after age and body mass index (BMI) adjustments. The detected interactions between the COMT Met and IL-10 A alleles, as well as between the COMT Met and TNF-α T alleles were insignificant after age and BMI adjustments. **Conclusion.** COMT and IL-1α may be used as candidate genes for early-onset PE and its severe form and small for gestational age (SGA) complications.

Key words:
comt protein, human; cytokines; pre-eclampsia; polymorphism, genetic.

Apstrakt

Uvod/Cilj. Preeklampsija pripada grupi hipertenzivnih poremećaja u trudnoći sa prosečnom incidencom od 2,16% i predstavlja jedan od vodećih uzroka morbiditeta i mortaliteta majki i novorođenčadi širom sveta. Cilj ove studije bio je da se ispita potecijalna povezanost između polimorfizma Val158Met katehol-O-metiltransferaze (COMT), faktor nekroze tumora-alfa (TNF-α) -857 C>T, receptora 1 za faktor nekroze tumora (TNFR1) 36 A>G, interleukin-1 alfa (IL-1α) 4845 G>T i interleukin-10 (IL-10) –1082 A>G sa rizikom od pojave rane preeklampsije (PE) i njenih komplikacija. **Metode.** Ova studija obuhvatila je 47 bolesnica sa ranom PE, grupisanih prema težini oboljenja i prema veličini za odgovarajuću gestacionu starost, i 47 zdravih osoba. Polimorfizam Val158Met je genotipiziran analizom *polymerase chain reaction – restriction fragment lenght polymorphism* (PCR-RFLP), a polimorfizam inflamatornih citokina Sangerovom metodom sekvencioniranja. **Rezultati.** Aleli COMT Met i IL-1α T pokazali su protektivnu ulogu, smanjujući rizik rane PE nakon korekcije za starost i indeks telesne mase (BMI). Uočena interakcija između alela COMT Met i IL-10 A, kao i između alela COMT Met i TNF-α T nije bila statistički značajna nakon korekcije za starost i BMI. **Zaključak.** COMT i IL-1α se mogu koristiti kao geni kandidati za otkrivanje rane PE i njenih komplikacija, teškog oblika rane PE i PE sa zastojem u rastu.

Ključne reči:
comt protein, humani, citokini; preeklampsija; polimorfizam, genski.

Correspondence to: Tijana Krnjeta, Roche d.o.o. Milutina Milankovića 11a, 11 000 Belgrade, Serbia. E-mail: krnjetatijana@gmail.com

Introduction

Preeclampsia (PE) belongs to the group of hypertensive disorders in pregnancy with global average incidence of 2.16%. It is considered as one of the leading causes of maternal and neonatal morbidity and mortality worldwide [1–3]. Special attention should be paid to the early and severe form of PE, due to its correlation with a very high morbidity rate and a frequent occurrence of serious health complications [2, 4].

The mechanism that initiates PE development is still unknown. The hypothesis is that combined over-expressed inflammatory response and angiogenic imbalance potentially cause an endothelial dysfunction [5]. By analyzing epidemiological data, it has been emphasized that genetic factors are one of the main risk factors for PE development, and numerous candidate gene studies and linkage analyses have been carried out in this area [6].

Recently, one of the genes whose expression showed to have potential as a candidate gene for PE and could be connected with angiogenic imbalance is the COMT gene [7]. COMT is among the major enzymes responsible for the inactivation of catechol-estrogens, which play an important role in pregnancy management and fetal development [8]. Different studies showed a correlation between the Val158Met COMT polymorphism and increased risk of PE in different patient groups [9]. A more detailed investigation showed that fetal Val158Met COMT polymorphism was correlating with increased risk of PE, and maternal Val158Met COMT polymorphism showed a protective role [10]. Furthermore, in line with the theory of excessive inflammatory response, several polymorphisms of inflammatory cytokines showed to be associated with PE [11–13].

Recently we demonstrated that material COMT Met-Met genotype was associated with decreased risk of early-onset PE including its severe form as well as risk of small-for-gestational-age (SGA) complicating PE [14]. In contination of this study we investigated examine the potential correlation between the Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G polymorphisms and the risk of early-onset PE, the risk of a severe form of early-onset PE and risk of SGA complicating early-onset PE. Furthermore, the investigation included the assessment of potential interaction between associating polymorphisms in order to determine their potential synergistic or antagonistic effect.

Methods

Subjects

The study was conducted at the Clinic of Gynecology and Obstetrics, Clinical Centre of Serbia, Belgrade, in the period between September 2012 and December 2013. The official approval for this study was obtained from the Ethics Committee of the Clinical Centre of Serbia. All patients and control subjects were informed beforehand about this study and they provided their written informed consent to participate.

The study included two groups of participants: the group of 47 early-onset PE patients and the control group of 47 healthy cases. The early-onset PE group was divided into two subgroups: severe early-onset PE of 33 patients and mild early-onset PE of 14 patients. Based on the second criterion, all 47 early-onset PE patients were divided into two subgroups: appropriate-for-gestational-age (AGA) subgroup with 12 patients and SGA subgroup with 35 patients.

PE, early-onset PE and severe PE were defined according to the American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy [15]. SGA and AGA were defined according to the national birth weight distribution of the Serbian population [16]. The excluding criteria were any of the following: pregnant women with known abnormal fetal karyotype or chromosomal abnormalities, multi-fetal gestation, gestational hypertension without proteinuria, chronic hypertension, diabetes mellitus, cardiovascular disease, autoimmune disease and renal disease.

The control subjects were defined as healthy singleton pregnancies, having come to the Clinical Centre of Serbia for delivery, and delivering a healthy neonate at term (37 weeks of gestation or more) without medical or obstetric complications.

Antenatal care was provided according to hospital guidelines and protocols. One aliquot of analyzed ethylenediaminetetraacetic acid (EDTA) whole blood was kept frozen on -70°C for deoxyribonucleic acid (DNA) extraction and genotyping. At delivery, the type of delivery was recorded; gestational age was calculated; birth weight was measured, and the Apgar score was assessed.

DNA preparation and genotyping

Isolation of genomic DNA from 200 μL of peripheral blood was done with the commercial kit for isolating genomic DNA (Roche Diagnostics), in accordance with the manufacturer's instructions. The detection of mutation presence in the gene for inflammatory cytokines and Val158Met COMT was performed by a polymerase (PCR) chain reaction amplification of DNA. The amplification was carried out in a PCR instrument (Termocycler-in) GeneAmp PCR System 9700 (Applied Biosystems). The product of PCR reactions for inflammatory cytokine polymorphism was than digested by EXOsap-IT enzyme. The Sanger sequencing method was used to analyze the expected polymorphism by the Genetic Analyzer Applied Biosystem 3130. Sequences were analyzed in the Software Sequencing Analysis 5.2 with a 36 cm capillary and polymer POP7. The product of PCR reactions for COMT polymorphism was digested by enzyme NlaIII (Hin1II Thermo SCENTIFIC). Electrophoretic separation was performed on 2.5% agarose gel, containing ethidium bromide. After digestion the enzyme fragments were visualized under ultraviolet light on transillumination (Wilber Lourmat).

Statistical analysis

General clinical characteristics between cases and controls were compared using Student's *t*-tests or the Wilcoxon rank sum test where appropriate. Genotype frequencies were

tested against theoretical Hardy-Weinberg equilibrium (HWE) by χ^2 contingency table analysis (degree of freedom = 2). Allele and genotype frequencies were compared between all cases of PE and their controls by contingency tables or by the Fisher's exact probability test, and odds ratios (OR) and 95% confidence intervals (CI) were computed. The frequency of homozygote for the common allele was considered as the reference for comparisons (OR = 1). Under a dominant model and a rare allele frequency of 0.34, our study sample had a power $1-\beta = 0.78$ to detect a genetic effect resulting in an OR = 0.2 at a type I error of 0.05. Power calculations were performed using the online tool Genetic Power Calculator [17].

The polymorphisms individually associated with PE (with $p < 0.05$ as entry criteria) were included in logistic regression models to test for joint multi-locus association with PE, both unadjusted and adjusted for clinical co-variables.

All the computation was done in R language and environment, version 3.1.0 [18].

Results

Clinical characterization

As shown in the previous study [14] significant differences were observed between early-onset PE patients and the control group in maternal age, body mass index (BMI), systolic and diastolic blood pressure (BP) and in gestational age at delivery. There was a significantly higher risk of SGA neonate delivery in patients with early-onset PE (Table 1). Deviataion from Hardy-Weinberg equlibrium (HWE) – D values for interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α) and tumor necrosis factor receptor type I (TNFRI) were significant in investigated loci. In contrast, genotypes for COMT and interleukin-1 alpha (IL-1α) in the investigated population respectively comply with the HWE proportions (Table 2).

COMT Val158Met genotyping

The Met allele decreased the risk for early-onset PE development and early-onset PE SGA development. There was no statistically significant difference between the mild and severe form of early-onset PE (Table 3). The strongest statistically significant influence was noticed in the allele recessive model. The MetMet genotype was decreasing the risk of early-onset PE and PE complications (Table 4).

IL-1α4845 G>T genotyping

T allele decreased the risk for early-onset PE development, severe early-onset PE and early-onset PE SGA development (Table 5). GT and TT genotypes were associated with decreased risk of early-onset PE and PE complications (Table 6).

IL-10-1082 A>G, TNF-α -857 C>T and TNFR1 36 A>G genotyping

There was no statistically significant association between IL -1082 A>G, TNF-α -857 C>T or TNFR1 36 A>G genotype and early-onset PE, severe early-onset PE or early-onset PE SGA observed ($p > 0.05$).

Multinomial logistic regression x̄

Older age and increased BMI significantly led to early-onset PE development. Even after age and BMI correction, a

Table 1

Clinical characteristics of examined patients and controls

Characteristics of patients	Controls n = 47, x̄ ± SD	Early-onset PE n = 47, x̄ ± SD (*p*)	Severe early-onset PE n = 33, x̄ ± SD (*p*)	Mild early-onset PE n =14, x̄ ± SD (*p*)	Early-onset PE SGA n = 35, x̄ ± SD (*p*)	Early-onset PEAGA n = 12, x̄ ± SD (*p*)
Age (years)	29.44 ± 4.49	32.14 ± 5.52 (0.009767)	32.45 ± 5.22 (0.007997)	31.42 ± 6.3 (0.2244)	32.06 ± 6.09 (0.03021)	32.41 ± 3.55 (0.03643)
Gestational age (days)	275 ± 9	225 ± 28 (1.346×10^{-14})	218 ± 23 (3.142×10^{-15})	241 ± 33 (2.398×10^{-5})	219 ± 24 (5.357×10^{-14})	240 ± 34 (2.119×10^{-6})
BMI (kg/m^2)	24.16 ± 4.11	27.77 ± 3.93 (0.0002266)	27.9 ± 4.25 (0.0007854)	27.46 ± 3.18 (0.01384)	27.0 ± 3.37 (0.003876)	30.03 ± 4.71 (0.0007481)
Systolic BP (mmHg)	108.62 ± 9.9	162.98 ± 18.61 (3.456×10^{-13})	171.36 ± 15.07 (1.037×10^{-11})	143.21 ± 8.23 (1.758×10^{-7})	163.57 ± 18.92 (8.828×10^{-12})	161.25 ± 18.35 (4.39×10^{-7})
Diastolic BP (mmHg)	70.17 ± 8.5	104.36 ± 11.68 (3.898×10^{-13})	109.24 ± 9.36 (9.147×10^{-12})	92.86 ± 8.02 (3.333×10^{-7})	104.57 ± 11.14 (7.54×10^{-12})	103.75 ± 13.67 (8.559×10^{-7})
Proteinuria (g/24 h)	/	3.74 ± 4.05	4.59 ± 4.41	1.73 ± 2.0	4.31 ± 4.12	2.07 ± 3.49
Birth weight (g)	3340.24 ± 445.28	1511.3 ± 784.2 (1.853×10^{-13})	1304.5 ± 536.2 (1.918×10^{-13})	1998.6 ± 1050.8 (8.639×10^{-5})	1206.3 ± 450.3 (7.715×10^{-14})	2400.8 ± 886.5 (0.0005678)

PE – preeclampsia; BMI – body mass index; SGA – small for gestational age; AGA – appropriate for gestational age; BP – blood pressure.

Table 2

Testing for Hardy-Weinberg Equilibrium (HWE)

HWE in the control group (n = 47)	Deviation from HWE (D)	χ^2	*p*
COMT	0.032	2.1942	0.3338
IL-1α	0.174	0.805	0.6686
IL-10	0.214	0.0474	1
TNF-α	0.215	0.0686	1
TNFRI	-0.219	0.0504	1

IL-1α – interleukin-1 alpha; IL-10 – interleukin-10; TNF-α – tumor necrosis factor-alpha; TNFRI – tumor necrosis factor receptor type I; COMT – catechol-o-methyltransferase.

Table 3

Distribution of COMT alleles in the investigated group of early-onset preeclampsia (PE) patients and the control group

COMT (allelic)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
Val	(37)	(52)	(37)	(15)	(39)	(13)
Met	(57)	0.526(42)*	0.511(29)	0.565(13)	0.518(31)**	0.552(11)

***p* = 0.04057; ****p* = 0.0411;**
SGA – small for gestational age; AGA – appropriate for gestational age; BP – blood pressure; COMT – catechol-o-methyltransferase; n – number.

Table 4

The COMT allele recessive model in the investigated group of early-onset preeclampsia (PE) patients and the control group

COMT (under AR assumption)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
Val-Val and Met-Val	(27)	(39)	(27)	(12)	(29)	(10)
Met-Met	(20)	0.281 (8)*	0.304 (6)**	0.229 (2)	0.284 (6)***	0.275 (2)

***p* = 0.01235, Fisher exact test; ****p* = 0.02928; *****p* = 0.01732;**
SGA – small for gestational age; AGA – appropriate for gestational age; BP – blood pressure; COMT – catechol-o-methyltransferase; n – number.

Table 5

Distribution of interleukin-1 alpha (IL-1α) alleles in the investigated group of early-onset preeclampsia (PE) patients and control group

IL-1α (allelic)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
G	(62)	(83)	(58)	(25)	(61)	(22)
T	(32)	0.259 (11)*	0.269 (8)**	0.235 (3)***	0.288 (9)****	0.178 (2)*****

***p* = 0.0004281; ****p* = 0.0016; *****p* = 0.0173; ******p* = 0.0019; *******p* = 0.012**
SGA – small for gestational age; AGA – appropriate for gestational age; OR – odds ratio; n – number.

Table 6

The interleukin-1 alpha (IL-1α) allele dominant model in the investigated group of early-onset preeclampsia (PE) patients and the control group

IL-1α (under AD assumption)	Controls (n)	Early-onset PE OR (n)	Severe early-onset PE OR (n)	Mild early-onset PE OR (n)	Early-onset PE SGA OR (n)	Early-onset PE AGA OR (n)
GG	(22)	(38)	(26)	(12)	(27)	(11)
GT and TT	(25)	0.212 (9)*	0.241 (7)**	0.151 (2)***	0.265 (8)****	0.082 (1)*****

***p* = 0.001123; ****p* = 0.00532; *****p* = 0.0136; ******p* = 0.0067; *******p* = 0.0075;**
SGA – small for gestational age; AGA – appropriate for gestational age; OR – odds ratio.

statistically significant association between Val158COMT or IL-1α polymorphism and early-onset PE development still remained.

COMT-Met homozygous was showing a protective role by reducing the risk for early-onset PE development 3.2 times, as well as IL-1αT allele, of which one dose reduced the risk for early-onset PE development for almost six times (Table 7).

Interactions between Val158Met COMT, TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G polymorphisms

The presence of COMT Met allele and TNF-α T allele additionally increased the risk for early-onset PE development 2.765-fold in comparison to the simple multiplying of both OR. After age and BMI adjustment, this interaction be-

Table 7

Multinomial logistic regression including age, body mass index (BMI), COMT and interleukin-1 alpha (IL-1α) polymorphisms

Variable / polymorphism	Early-onset PE adjusted OR (95% CI)	Early-onset PE *p*-value
Age (years)	1.122(1.015–1.253)	0.030064
BMI (kg/m^2)	1.134(1.133–1.568)	0.000876
COMT (AR model)	0.308(0.091–0.960)	0.047627
IL-1α (AD model)	0.167(0.048–0.508)	0.002620

PE – preeclampsia; OR – odds ratio; CI – confidence intervals; BMI – body mass index; IL-1α – interleukin-1 alpha; COMT – catechol-o-methyltransferase.

came statistically insignificant. The interaction between COMT Met allele and IL-10 A allele was close to the statistical level of significance, uncorrected as well as corrected for age and BMI. Combined OR was significantly different (almost three times higher) in comparison to the multiple of those two OR, leading to the conclusion that IL-10 A allele presence reduced the protective effect of COMT Met allele 2.71-fold (Table 8).

Table 8

Interactions of COMT and inflammatory cytokines polymorphisms

Interactions	OR for interaction term – unadjusted for BMI and age	*p* – unadjusted for BMI and age	OR for interaction term, adjusted for BMI and age	*p* – adjusted for BMI and age	OR combined, adjusted for BMI and age
COMT (Met) : IL-1α (T)	0.618	NS	0.509	NS	0.190
COMT (Met):IL-10 (A)	0.369	0.0647	0.357	0.0827	0.937
COMT(Met):TNF-α(T)	2.765	0.0491	1.703	NS	0.490
IL-1α(T):IL-10(A)	0.799	NS	1.046	NS	0.159
IL-1α(T):TNF-α(T)	1.948	NS	2.486	NS	0.371
IL-10(A):TNF-α(T)	1.724	NS	1.470	NS	1.239

BMI – body mass index; IL-1α – interleukin-1 alpha; IL-10 – interleukin-10; TNF-α – tumor necrosis factor-alpha; COMT – catechol-o-methyltransferase; OR – odds ratio.

Another group of polymorphisms that were included in the scope of our investigation are inflammatory cytokine gene polymorphisms: TNF-α -857 C>T, TNFR1 36 A>G, IL-1α 4845 G>T and IL-10 -1082 A>G. Haggerty et al. [11] showed that IL-1α 4845 GG genotype significantly increased the PE risk (black OR 11,6; 95% CI 1,5-89,3; white OR 1,7; 95% CI 0,7–3,9). The combination of TNF-α -857C>T and TNFR1 36 A>G polymorphisms leads to 2.26-fold times increased risk of PE. The effect was even stronger in their joint presence with IL-1α 4845 G>T polymorphism, which together were associated with higher than 4 times higher risk of PE development (OR = 4.13; CI95: 2.16 – 7.89; p = 0.00002) [12]. The most frequently investigated polymorphism was IL-10 -1082 A>G. However, a meta-analysis performed by Lee et al. [13] showed a statistically insignificant association, after excluding the studies where the distribution of control subjects was deviating from HWE. In our study, we could only find a statistically significant association between IL-1α 4845 G>T polymorphism and the risk of early-onset PE, severe early-onset PE and early-onset PE with SGA. The potential reason could be the limited number of patients included in this study, as well as a potentially inappropriate control population selection for these polymorphisms, described by significant deviation from HWE for TNF-α, TNFR1and IL-10 in the control group.

Regarding the potential of the IL-1α 4845 G>T polymorphism to do the risk stratification of PE, to our knowledge this is the first study investigating this topic. Significant differences in the IL-1α 4845 G>T allele distribution between the mild and severe form of PE could be identified. We also showed the association between the IL-1α 4845 G>T polymorphism and early-onset PE associated with SGA.

For the statistically significant loci, adjustments for age and BMI was made in a multivariate logistic regression analysis. It is important to underline that we confirmed previously published data about positive association between

Discussion

Many genes and their polymorphisms have been examined in order to detect the potential markers for high-risk pregnancies [19, 20]. Due to the most recent highlights in the theories of 'excessive inflammation' and 'angiogenic imbalance' as the potential causes of PE, there is a dilemma whether the polymorphisms of genes associated with these could be a potential cause of PE development [5].

Recently, the animal COMT mice knockout model has shown to be useful in clarifying the significance of decreased COMT expression in PE [7]. Even though this study was revolutionary in the field of PE genetic models investigation, it was clearly limited with the absence of differentiation between maternal and fetal low COMT activity. Our study confirmed the protective role of low maternal COMT activity. The explanation could be found in a hypothesis that Hill et al. [10] proposed, which considered decreased maternal COMT activity as a protective role by stimulating the placenta to produce 2-ME. Placental low COMT activity is the key contributor for PE development. We are further supporting our finding with the fact that our control group is on HWE for COMT genotype.

Regarding the potential association of COMT allele distribution and early-onset PE complicated with SGA, our study showed that MetMet decreased the risk of early-onset PE SGA for more than 3.5 times. To the contrary, Sata et al. [21] showed that patients with homozygous COMT-L alleles had a 2.98 times higher risk of low birth weight (< 2,500 g) [20]. It is concluded that lower COMT activity may lead to the accumulation of catechol estrogens, and thus, cause oxidative DNA damage which may be associated with SGA [22]. These explanations could be applied to fetal low COMT activity and our suggestion is that low maternal COMT activity could show a protective role allowing higher fetal COMT activity. Further studies are needed to investigate these contradictory data.

age or BMI with PE development [23]. Even after age, BMI and other statistically significant polymorphism adjustments, each of the two polymorphisms still showed a statistically significant association with early-onset PE development.

Finally, we investigated the interactions between the previously mentioned polymorphisms and their association with early-onset PE, which is to our knowledge the first study investigating this topic. The allele model showed a statistically significant interaction between the COMT Met allele and TNF-α T allele which significance after age and BMI adjustments disappeared. Secondly, the interaction between COMT Met and IL-10 A alleles, unadjusted as well as age and BMI adjusted, were on the level of statistical significance. Further studies are needed in order to investigate this suggestion in the larger population size.

The main shortcoming of our study was the limited number of investigated patients, as we were primarily focused on difficult cases of early-onset PE (33 severe early-onset PE patients in comparison to 47 early-onset PE patients in total) with a mean systolic BP 162.98 ± 18.61 mmHg and diastolic BP 104.36 ± 11.68 mmHg. Further studies with a bigger sample size of early-onset PE patients should be conducted in order to investigate the significance of a potential interaction between COMT and inflammatory cytokines polymorphisms.

Conclusion

We showed that the distribution of COMT as well as IL-1α alleles between early-onset PE patients and control subjects was different, particularly between SGA early-onset PE patients and respected control subjects. The COMT Met allele as well as IL-1α T showed a protective role, decreasing the risk of early-onset PE after age and BMI adjustments. Interaction between COMT Met and IL-1α T did not show any statistically significance. Statistical significance was observed in interaction between the COMT Met and IL-10 A alleles, but it was not any more statistically significant after age and BMI adjustments. Interaction between the COMT Met and TNF-α T alleles were close to level of significance, both before and after age and BMI adjustments.

COMT and IL-1α may be used as candidate genes for detection of high-risk patients for development of early-onset PE and its severe form and SGA complications.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia on the basis of the contract No.175036.

The authors report no conflict of interest.

R E F E R E N C E S

1. *Abalos E, Cuesta C, Carroli G, Qureshi Z, Widmer M, Vogel JP.* WHO Multicountry Survey on Maternal and Newborn Health Research Network. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: A secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG 2014; 121 Suppl 1: 14−24.
2. *Say L, Chou D, Gemmill A, Tunçalp Ö, Moller AB, Daniels J,* et al. Global causes of maternal death: A WHO systematic analysis. Lancet Glob Health 2014; 2(6): e323−33.
3. *Alkema L, New JR, Pedersen J, You D.* UN Inter-agency Group for Child Mortality Estimation; Technical Advisory Grpup. Child mortality estimation 2013: An overview of updates in estimation methods by the United Nations Inter-agency Group for child mortality estimation. PloS ONE 2014; 9(7): e101112.
4. *Srinivas SK, Edlow AG, Neff PM, Sammel MD, Andrela CM, Elovitz MA.* Rethinking IUGR in preeclampsia: Dependent or independent of maternal hypertension. J Perinatol 2009; 29(10): 680−4.
5. *Ramma W, Ahmed A.* Is inflammation the cause of pre-eclampsia. Biochem Soc Trans 2011; 39(6): 1619−27.
6. *Valenzuela FJ, Perez-Sepulveda A, Torres MJ, Correa P, Repetto GM, Illanes SE.* Pathogenesis of preeclampsia: The genetic component. J Pregnancy 2012; 2012: 632732
7. *Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L,* et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature 2008; 453(7198): 1117−21.
8. *Zhu BT, Wu KY, Wang P, Cai MX, Conney AH.* O-methylation of catechol estrogens by human placental catechol-o-methyltransferase: interindividual differences in sensitivity to heat inactivation and to inhibition by dietary polyphenols. Drug Metab Dispos 2010; 38(10): 1892−9.
9. *Lim JH, Kim SY, do Kim J, Park SY, Han HW, Han JY,* et al. Genetic polymorphism of catechol-O-methyltransferase and cytochrome P450c17a in preeclampsia. Pharmacogenet Genomics 2010; 20(10): 605−10.
10. *Hill LD, York TP, Kusanovic JP, Gomez R, Eaves LJ, Romero R,* et al. Epistasis between COMT and MTHFR in maternal-fetal dyads increases risk for preeclampsia. PLoS One 2011; 6(1): e16681.
11. *Haggerty CL, Ferrell RE, Hubel CA, Markovic N, Harger G, Ness RB.* Association between allelic variants in cytokine genes and preeclampsia. Am J Obstet Gynecol 2005; 193(1): 209−15.
12. *Laroche M, Girouard J, Forest JC, Rousseau F, Giguère Y.* A polygenic model of susceptibility to preeclampsia: Dose-effect of genes involved in the inflammatory process. Available from: theses.ulaval.ca/archimede/fichiers/26217/ch03.html
13. *Lee YH, Kim JH, Song GG.* Meta-analysis of associations between interleukin-10 polymorphisms and susceptibility to pre-eclampsia. Eur J Obstet Gynecol Reprod Biol 2014; 182: 202−7.
14. *Krnjeta T, Mirković L, Ignjatović S, Tomašević D, Lukić J, Topalov D,* et al. Protective Role of Maternal P.VAL158MET Catechol-O-Methyltransferase Polymorphism against Early-Onset Preeclampsia and its Complications. J Med Biochem 2016; 35(3): 3128.
15. *American College of Obstetricians and Gynecologists. Task Force on Hypertension in Pregnancy.* Hypertension in Pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 2013; 122(5): 1122−31.
16. Statistical Office of the Republic of Serbia, United Nations Children's Fund. Multiple Indicator Cluster Survey, Monitoring the situation of children and women. Belgrade: Unicef; 2010.

17. *Purcell S, Cherny SS, Sham PC.* Genetic Power Calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003; 19(1): 149−50.
18. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014. Available from: http://www.R-project.org/
19. *Goddard KA, Tromp G, Romero R, Olson JM, Lu Q, Xu Z,* et al. Candidate-gene association study of mothers with preeclampsia, and their infants, analyzing 775 SNPs in 190 genes. Hum Hered 2007; 63(1): 1−16.
20. *Carreiras M, Montagnani S, Layrisse Z.* Preeclampsia: A multifactorial disease resulting from the interaction of the feto-maternal HLA genotype and HCMV infection. Am J Reprod Immunol 2002; 48(3): 176−83.
21. *Sata F, Yamada H, Suzuki K, Saijo Y, Yamada T, Minakami H,* et al. Functional maternal catechol-O-methyltransferase polymorphism and fetal growth restriction. Pharmacogenet Genomics 2006; 16(11): 775−81.
22. *Scholl TO, Stein TP.* Oxidant damage to DNA and pregnancy outcome. J Matern Fetal Med 2001; 10(3): 182−5.
23. *English FA, Kenny LC, McCarthy FP.* Risk factors and effective management of preeclampsia. Integr Blood Press Control 2015; 8: 7−12.

Received on March 29, 2016.
Revised on April 08, 2016.
Accepted on April 11, 2016.
Online First October, 2016.