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PROTECTIVE ROLE OF MATERNAL P.VAL158MET CATECHOL-O-METHYLTRANSFERASE POLYMORPHISM AGAINST EARLY-ONSET PREECLAMPSIA AND ITS COMPLICATIONS

ZAŠTITNA ULOGA POLIMORFIZMA P.VAL158MET KATEHOL-O-METILTRANSFERAZA KOD MAJKE U NASTANKU RANE PREEKLAMPSIJE I NJENIH KOMPLIKACIJA

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Summary

Background: Up until now there have been contradictory data about the association between p.Val158Met catechol-O-methyltransferase (COMT) polymorphism and risk of preeclampsia (PE). The goal of this study was to assess the potential correlation between p.Val158Met *COMT* polymorphism and risk of early-onset PE, risk of a severe form of early-onset PE, as well as risk of small-for-gestational-age (SGA) complicating PE.
Methods: The study included 47 early-onset PE patients and 47 control cases. Forty-seven early-onset PE patients were grouped by disease severity (33 patients with a severe form and 14 patients without severe features) and secondly by size for gestational age (12 patients with appropriate-for-gestational-age (AGA) and 35 patients with SGA size). p.Val158Met polymorphism was genotyped by PCR-RFLP analysis.
Results: Allele analysis showed significant difference in *COMT* allele distribution between early-onset PE and con-

Kratak sadržaj

Uvod: Do sada su postojali kontradiktorni podaci o povezanosti polimorfizma p.Val158Met katehol-O-metiltransferaza (COMT) i rizika od pojave preeklampsije (PE). Cilj ove studije je bio da se utvrdi potencijalna povezanost između p.Val158Met *COMT* polimorfizma i rizika od razvoja rane PE, teškog oblika rane PE, kao i rane PE sa zastojem u rastu (SGA).
Metode: Studija je obuhvatila 47 pacijentkinja sa ranom PE i 47 kontrolnih subjekata. Četrdeset sedam pacijentkinja sa ranom PE su bile podeljene na osnovu težine oboljenja (33 pacijentkinje sa teškim oblikom i 14 pacijentkinja sa odsustvom teškog oblika) i sekundarno na osnovu veličine za odgovarajuću gestacionu starost (12 pacijentkinja sa odgovarajućom veličinom novorođenčadi za gestacionu starost i 35 pacijentkinja sa manjom veličinom novorođenčadi za gestacionu starost). Polimorfizam p.Val158Met je određen analizom PCR-RFLP.

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List of abbreviations: COMT (catechol-O-methyltransferase), PE (preeclampsia), SGA (small-for-gestational-age), AGA (appropriate-for-gestational-age), HELLP (a combination of the breakdown of red blood cells [hemolysis; the H in the acronym], elevated liver enzymes [EL], and low platelet count [LP] occurring in pregnancy), BMI (body mass index), BP (blood pressure), LDH (lactate dehydrogenase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), EDTA (ethylenediaminetetraacetic acid), DNA (deoxyribonucleic acid), PCR (polymerase chain reaction), HWE (Hardy-Weinberg equilibrium), OR (odds ratio), CI (confidence interval), 2-ME (2-methoxyestradiol), HIF-1α (hypoxia-inducible factor 1α), sFlt-1 (soluble fms-like tyrosine kinase 1), IUGR (intrauterine fetal growth restriction), CYP1A1 (cytochrome P450 1A1), GSTT1 (glutathione S-transferase T1), IGF-I (insulin-like growth factor-I).

trol group as well as early-onset PE SGA and controls (p=0.04057 and p=0.0411 respectively). A statistically significant distribution difference between the severe form and form without severe features of early-onset PE patients was not observed (p>0.05). The highest difference observed was in the allele recessive model where *COMT* MetMet genotype was associated with decreased risk of early-onset PE (OR=0.281; 95%CI=0.092–0.7836) and PE complications including severe early-onset PE (OR=0.304; 95%CI=0.086–0.944) and SGA early-onset PE (OR=0.284; 95%CI=0.081–0.874).

Conclusions: *COMT* may be used as a candidate gene for early-onset PE and its severe form and SGA complications.

Keywords: COMT, polymorphism, early-onset preeclampsia, severe, SGA

Rezultati: Alelska analiza pokazala je značajnu razliku u distribuciji *COMT* alela između pacijentkinja sa ranom PE i kontrola kao i pacijentkinja sa ranom PE i SGA i kontrola (p=0,04057, odnosno p=0,0411). Nije uočena statistički značajna razlika u distribuciji između teške forme PE i forme PE bez teških karakteristika (p>0,05). Najznačajnija razlika uočena je u alelskom recesivnom modelu gde je *COMT* MetMet genotip bio povezan sa smanjenim rizikom od pojave rane PE (OR=0,281; 95%CI=0,092–0,7836) i komplikacija PE uključujući tešku formu rane PE (OR=0,304; 95%CI=0,086–0,944) i rane PE komplikovane zastojem u rastu (OR=0,284; 95%CI=0,081–0,874).

Zaključak: *COMT* bi mogao da se koristi kao gen kandidat za pojavu rane PE i njenih komplikacija, teškog oblika i zastoja u rastu.

Ključne reči: COMT, polimorfizam, rana preeklampsija, teška, zastoj u rastu

Introduction

Preeclampsia (PE), a hypertensive disorder in pregnancy, affects 3–8% of pregnancies worldwide. It is the leading cause of maternal and fetal morbidity and mortality (1–6). The exact pathogenesis of PE is still unclear and different mechanisms have been proposed in order to clarify it more precisely (7, 8). 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 (9).

Recently, one of the genes whose expression showed potential as a candidate gene for PE has been the catechol-O-methyltransferase (COMT) gene (10). *COMT* is among the major enzymes responsible for inactivation of catechol-estrogens, which play an important role in pregnancy management and fetal development. One of the functional polymorphisms in the *COMT* gene is the presence of G instead of A base (rs4680) causing the positioning of the amino acid methionine instead of valine at codon 158, and thereby decreasing COMT activity (11, 12). Different studies showed association between p.Val158Met *COMT* polymorphism and an increased risk of PE in different patient groups (11, 13). More recently, it was shown that fetal p.Val158Met *COMT* polymorphism correlated with increased risk of PE, and maternal p.Val158Met *COMT* polymorphism showed a protective role (14).

According to the authors' knowledge, there is no study investigating the link between p.Val158Met *COMT* polymorphism and the increased risk of PE in the populations of the Balkan Peninsula, the Serbian population included. Also, there is no known study investigating the possible association between p.Val158Met *COMT* polymorphism and very early-onset PE. Early-onset PE seems to be a placenta-mediated complication and is associated with abnormal uterine artery Doppler flow, fetal growth restriction and adverse outcomes in mothers as well as in fetuses (15).

The aim of this study was to examine the potential correlation between p.Val158Met *COMT* polymorphism and the risk of early-onset PE, the risk of severe form of early-onset PE and risk of small-for-gestational-age (SGA) complicating PE in the Serbian population.

Materials and Methods

Subjects

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

In a total of 94 participants, there were two groups of patients: an early-onset PE group with 47 patients (50%) and 47 controls (50%). The early-onset PE group was divided into two subgroups: severe form of early-onset PE with 33 cases and mild form of early-onset PE with 14 cases. Six patients with HELLP syndrome were included in the severe form of early-onset PE subgroup. Based on the second criterion, all 47 early-onset PE patients were divided in two subgroups, the AGA subgroup with 12 patients and the SGA subgroup with 35 patients. Significant differences were observed between these two groups 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. Clinical characteristics of examined patients and controls can be seen in *Table I*.

PE, early-onset PE and severe PE were defined according to the American College of Obstetricians

Table I Clinical characteristics of examined patients and controls.

	Controls n=47	Early-onset PE n=47 (p)	Severe early-onset PE n=33 (p)	Mild early-onset PE n=14 (p)	Early-onset PE SGA n=35 (p)	Early-onset PE AGA n=12 (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/24h)	/	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)

and Gynecologists Task Force on Hypertension in Pregnancy (5). HELLP syndrome was defined when three of the following criteria were positive in the absence of other pathologic conditions: lactate dehydrogenase (LDH) > 600 U/L, aspartate aminotransferase (AST) > 70 U/L or alanine aminotransferase (ALT) > 70 U/L, and platelet count < 100,000 cells/mm (6).

SGA and AGA were defined as birth weight below the 10th percentile and birth weight between the 10th and 90th percentile, respectively, 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, multifetal 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 Center of Serbia for delivery, and delivering a healthy neonate at term (37 weeks of gestation or more) without medical or obstetric complications.

Antenatal care protocol

Antenatal care was provided according to hospital guidelines and protocols. At presentation, all patients gave a detailed medical history and underwent a general physical examination, which included detection of edema, new onset of cerebral or visual disturbances and presentation of pulmonary edema. BMI was calculated by dividing the weight (kg) by the square of height (m^2). BP was measured in the sitting position on the right arm after a 10-min rest, and the first and fifth phases were recorded. Gestational age was assessed based on ultrasound measurement. Laboratory analyses were done in the morning after at least 12 h of fasting. Creatinine, total bilirubin, LDH, AST and ALT were measured by colorimetric methods. Platelets were determined by impedance measurement. One aliquot of analyzed EDTA-whole blood was kept frozen at –70 °C for DNA extraction and genotyping (no additional blood collection was performed). Proteins in 24-h urine were measured by the colorimetric method, with Pyrogallol red. In cases where there was no time for 24-h urine collection or an inadequate procedure for 24-h urine collection was applied, a visual dipstick reading was performed. At delivery, the type of delivery was recorded; gestational age was calculated; birth weight was measured; and the Apgar score was assessed.

DNA extraction 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 manufacturer's instructions. The detection of variant presence in the gene p.Val158 Met *COMT* was performed by chain reaction amplification of DNA. It was carried out in a 25 µL mixture volume containing: a 12.5 µL QIAGEN Multiplex PCR

kit, 100 ng of isolated genomic DNA and 10 pmol sense and antisense primers. Sense (P1) and antisense (P2) primers were 5′-ACT GTG GCT ACT CAG CTGTG-3′ and 5′-CCT TTT TCC AGG TCT GAC AA-3′ respectively.

The amplification was carried out in a PCR instrument (Termocycler-in) GeneAmp PCR System 9700 (Applied Biosystems). Terms of PCR reactions were as follows: denaturation at 94 °C 5 min; 30 cycles of amplification consisting of denaturation at 94 °C for 30 s, primer binding (annealing) at 66 °C for 30 s and elongation at 72 °C for 1 min. The final elongation was carried out at 72 °C for 5 min. The product of PCR reactions consisted of 169 bp and included the investigated polymorphism p.Val158Met. The digestion amplification product, enzyme NlaIII (Hin1II Thermo SCENTIFIC), was used. Electrophoretic separation was performed on 2.5% agarose gel, containing ethidium bromide. In the case of the unmodified (wild type) Val allele, enzyme cuts the PCR product into fragment lengths of 114 bp, 29 bp and 26 bp. In the case of the variant allele, Met enzyme cuts the PCR product into fragment lengths of 96 bp, 29 bp, 26 bp and 18 bp. After digestion, the enzyme fragments were visualized under ultraviolet light transillumination (Wilber Lourmat).

Statistical analysis

General clinical characteristics between cases and controls were compared using Student's t-tests or Wilcox rank sum test where appropriate. Genotype frequencies were tested against the 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 homozygotes 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-β = 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 in the online tool Genetic Power Calculator (17).

Polymorphism was included in logistic regression models to be adjusted for clinical co-variables.

All the computation was done in R language and environment, version 3.1.0 (18).

Results

Genotype analysis indicated that the difference between the two investigated groups was at the conventional level of significance. In order to estimate allelic relative risks, allele-based parameterizations on risk parameters were proposed. Allelic expression analysis showed a statistically significant difference in allele distribution between early-onset PE, early-onset PE SGA and controls. In patients with early-onset PE, Met allele was associated with 1.9 times lower risk of developing early-onset PE, showing a protective role. Similar situation was noted with early-onset PE SGA. In patients with early-onset PE complicated by SGA, Met allele was associated with 1.93 times lower risk of early-onset PE SGA development. Regarding the

Table II Distribution of COMT alleles, COMT allele dominant model and COMT allele recessive model in the investigated group of early-onset preeclampsia patients and control group.

		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
COMT (genotype)	Wild type	(10)	(13)	(10)	(3)	(10)	(3)
	Heterozygous	(17)	1.173 (26)*	1 (17)	1.74 (9)	1.115 (19)	1.361 (7)
	Homozygous	(20)	0.315 (8)**	0.308 (6)	0.345 (2)	0.308 (6)	0.345 (2)
COMT (allelic)	Val	(37)	(52)	(37)	(15)	(39)	(13)
	Met	(57)	0.526 (42)***	0.511 (29)	0.565 (13)	0.518 (31)****	0.552 (11)
COMT (under AD assumption)	Val-Val	(10)	(13)	(10)	(3)	(10)	(3)
	Met-Met and Met-Val	(37)	0.709 (34)	0.625 (23)	0.991 (11)	0.679 (25)	0.814 (9)
COMT (under AR assumption)	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=0.797; ** p=0.052; ***p=0.04057; ****p=0.0411; *****p=0.01235, Fisher exact test; ******p=0.02928; *******p=0.01732

Table III Multinomial logistic regression including age, BMI and *COMT* 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	1.134 (1.133–1.568)	0.000876
COMT (AR model)	0.308 (0.091–0.960)	0.047627

allele distribution difference between the two subgroups of early-onset PE patients, mild and severe form of PE, there was no difference between these two subgroups at the conventional level of significance (*Table II*).

Further introducing the allelic dominant and the allelic recessive model, a more detailed analysis was applied. In the allelic dominant model, the Met-Met and Met-Val genotypes did not show any statistically significant distribution difference between early-onset PE, severe form of early-onset PE, early-onset PE SGA and control subjects. In the allelic recessive model, the *COMT* MetMet genotype showed a statistically significant distribution difference between early-onset PE, severe form of early-onset PE, early-onset PE SGA and controls. The *COMT* MetMet genotype showed a protective role, by decreasing the risk of early-onset PE and its complications, including severe early-onset PE and SGA early-onset PE. The risk was more markedly decreased for SGA early-onset PE and, generally, the most markedly decreased for early-onset PE (*Table II*).

Finally, *COMT* locus was investigated together with age and BMI in a multivariate logistic regression analysis for assessment of potential association with the total early-onset PE development. Age and BMI were associated with approximately 1.1 times higher risk of early-onset PE development. In *COMT* Met homozygous subjects a protective role was shown by reducing the risk for early-onset PE development 3.2 times. This effect remained even after age and BMI adjustment (*Table III*).

Discussion

Precise and early recognition of high risk patients may contribute to better management of PE patients. Special focus should be placed on early-onset PE, since the risk of adverse maternal and perinatal outcome increases significantly when preeclampsia develops early (19, 20). An epidemiological study (21) has indicated that PE has a strong genetic component to its occurrence, with additional geographic, socio-economic and racial risk factors. Many genes and their polymorphisms have been investigated as gene candidates for susceptibility to preeclampsia (22–24).

Recently, one of the genes whose expression showed the potential to be used as a PE candidate gene was a *COMT* gene (10). *COMT* is one of the key enzymes involved in catechol estrogen inactivation (25). During pregnancy, significant increase in catechol estrogen production has been detected, potentially due to the increased activity of the placenta. Placenta shows high activity of estrogen 2-hydroxylase which produces 2-hydroxyestradiol and 4-hydroxyestradiol and the COMT enzyme which converts them into 2-metoxyestradiol (2-ME). In the presence of COMT, 2-ME suppresses hypoxia-inducible factor 1α (HIF-1α) accumulation and the production of soluble fms-like tyrosine kinase 1 (sFlt-1) (26, 27). It was suggested that during the PE, COMT is showing lower activity which leads to the lower production of 2-ME, accumulation of HIF-1α and increased production of sFlt-1 (28). Recently, the animal COMT mice knockout model has been shown to be useful in clarifying the significance of decreased *COMT* expression in PE. In *COMT-/-* mice increased concentration of HIF-1α induces an increased production of sFlt-1 thus causing an inflammatory reaction and endothelial dysfunction (10). A functional single-nucleotide polymorphism which codes the synthesis of membrane-bound COMT results in a valine to methionine variant at position 158 (p.Val158Met) rs4680 and decreased COMT activity (29). Different studies that have been recently published showed a correlation between *COMT* genotypes and the risk of developing PE in different populations. Lim et al. (13) and Liang et al. (11) showed association between *COMT* p.Val158Met polymorphism and an increased risk of PE and SGA in the Korean and Asian population respectively. Regarding available data related to European populations, Roten et al. (30) showed a correlation between p.Val158Met genotype and recurrent PE. However, a link between the p.Val158Met genotype and non-recurrent PE was not shown. Recently, in a more detailed analysis, Hil et al. (14) showed that maternal ACCG haplotype which is associated with lower COMT activity was in correlation with decreased PE risk. Explanation could be found in the hypothesis proposed by Hill et al. (14) which considers the idea that decreased maternal COMT activity has a protective role by stimulating the placenta to produce 2-ME. Placental low COMT activity is the key contributor to PE development. In our study and the targeted subject-patient population, the *COMT* Met-Met genotype in the allelic recessive model showed a protective role by decreasing the risk of early-onset PE 3.2-fold. This effect remained even after age and BMI adjustment.

Regarding the potential of the p.Val158Met genotype to do the risk stratification of PE, thus to differentiate between mild and severe forms, there are only limited data available. This study showed that

pregnancies with MetMet genotype had a lower risk of developing severe forms of PE. Contrary to this, Lim et al. (13) showed that the MetMet genotype was associated with an increased risk of developing a severe form of PE. Liang et al. (11) could not find statistical significance between severe and mild PE genotype and allele distribution. Our data are further supported by the distribution of *COMT* genotype which is in HWE. Further studies with bigger sample size are necessary to clarify the contradictory data.

Further interesting and promising data could be found regarding the potential association of *COMT* genotype with SGA PE or SGA itself. There is growing evidence that maternal and fetal genetic factors may play an important role in SGA development. Cytochrome P450 1A1 gene (CYP1A1), glutathione S-transferase T1 (GSTT1) and insulin-like growth factor-I (IGF-I) were some of the proposed genetic factors whose polymorphisms were found to be associated with SGA (31, 32). In this study, we showed that SGA early-onset PE patients have significantly different *COMT* allele distribution in comparison to control subjects. In the recessive allelic model, MetMet decreased the risk of SGA early-onset PE 3.52 times. Sata et al. (33) showed opposite data, that patients with homozygous *COMT-L* alleles had 2.98 times higher risk of low birth weight (<2500 g). It was concluded that lower COMT activity might lead to the accumulation of catechol estrogens due to its inability to inactivate them, and, consequently, cause oxidative DNA damage (34–36). Oxidative DNA damage at the end of pregnancy might be associated with SGA (37). Possible explanation of our data could be the consideration that decreased maternal COMT activity has a protective role by stimulating the placenta to produce 2-ME. Placental low COMT activity is the key contributor to PE development.

The main weakness of our study was the limited number of investigated patients, but we were primarily focused on the difficult cases of early-onset PE (33 severe early-onset PE patients in comparison to 47 early-onset PE patients in total) with systolic BP 162.9±19.0 mm Hg and diastolic BP 104.6±11.9 mm Hg.

Our data support the hypothesis established by Hill et al. (14) in an early-onset PE patient population. Further prospective studies in larger and ethnically diverse populations are needed in order to confirm the hypothesis as well as to identify the mechanisms behind it (38).

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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