ORIGINAL ARTICLE: GENETICS

Fetal Val108/158Met catechol-*O*-methyltransferase (COMT) polymorphism and placental COMT activity are associated with the development of preeclampsia

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Objective: To evaluate the association between fetal and maternal catechol-*O*-methyltransferase (COMT) Val158Met and methyl tetrahydrofolate reductase (MTHFR) C677T functional polymorphisms and preeclampsia, examining its influence on placental COMT and in maternal 2-methoxyestradiol (2-ME) plasma levels.
Design: Prospective case-control study.
Setting: University hospital.
Patient(s): A total of 53 preeclamptic and 72 normal pregnant women.
Intervention(s): Maternal and cord blood samples and placental tissue samples were obtained.
Main Outcome Measure(s): Maternal and fetal COMT and MTHFR polymorphisms were genotyped. Maternal plasma 2-ME and homocysteine levels, and expression and activity of placental COMT were measured.
Result(s): The odds ratio for the risk of preeclampsia for fetal COMT Met/Met was 3.22, and it increased to 8.65 when associated with fetal MTHFR TT. Placental COMT activity and expression were influenced by genotype, but COMT activity in preeclamptic placentas did not differ from control pregnancies. There was no association between any genotypes and maternal 2-ME. Homocysteine levels were higher in women with preeclampsia than in normal pregnancies, and were inversely correlated with 2-ME plasma levels, indicating that its altered metabolism may lower COMT activity in vivo.
Conclusion(s): Fetal Met-Met COMT genotype reduces COMT placental expression and activity in vitro and increases preeclampsia, risk but it does not explain the difference in maternal 2-ME levels between preeclamptic and normal pregnancies. However, the preeclamptic patients had elevated homocysteine levels that correlated inversely with 2-ME, indicating that an altered methionine-homocysteine metabolism may contribute to reduce COMT activity in vivo and explain the decreased levels of 2-ME in preeclamptic women. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: 2-Methoxyestradiol, preeclampsia, COMT, genotype, MTHFR

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Preeclampsia (PE) is one of the leading causes of maternal and fetal morbidity and mortality, affecting 5%–8% of pregnant women worldwide (1). The pathogenesis of this disease is still not completely understood. Recent evidences suggest that a deficiency in catechol-*O*-methyltransferase (COMT) is associated with

PE (2, 3), because COMT is a key enzyme in the metabolization of estrogens (4). 2-Methoxyestradiol (2-ME) is generated by hydroxylation at the 2-position of 17β-E_2 by cytochrome P450 enzymes, and the subsequent O-methylation of the catechol ring by COMT (5). Normal 2-ME plasma levels in women are in the picomolar range and increase more than 1,000-fold during pregnancy in women, reaching a plateau during the third trimester (6). Increased 2-ME levels during pregnancy are thought to be necessary for cytotrophoblast invasion of the maternal decidua, and its deficiency might be involved in the altered placentation present in PE (7). Recently, we have reported lower plasmatic 2-ME in PE than in normal pregnancies, and also found a significant association between lower plasma levels of 2-ME and clinical indexes of severity of the disease (8). Kanasaki et al. described that both circulating concentrations of 2-ME and placental COMT activity were significantly reduced in women diagnosed with PE, raising the possibility that altered production of 2-ME may contribute to the pathophysiology of PE by altering placental vascularization and its response to hypoxia (2).

In mammals, COMT is present in two molecular forms: a soluble cytoplasmic form (S-COMT) and a membrane protein (MB-COMT) (9) with similar kinetic mechanism. The main difference is the lower Michaelis constant (K_M) value for MB-COMT for catecholamines and higher maximum reaction rate (V_{max}) for S-COMT from human brain (10) and from cells containing recombinant S- or MB-COMT (11). This makes S-COMT the predominant isoform of the enzyme, especially at high concentrations of substrate (i.e., during pregnancy). However, the mechanisms linking human COMT to PE remain unclear. It is not entirely known, for example, why both lower concentrations and lower activity of COMT have been noted in human preeclamptic placentas. A possible explanation involves a common polymorphism in the gene encoding for COMT in exon 5 (substitution of valine [Val] for methionine [Met] at codon 108 for the S-COMT and at 158 for the MB-COMT; rs4680) (12), resulting in reduced thermostability and activity of the enzyme (9). An association between the functional Val158Met polymorphism and PE has been reported, but most of the studies derived only from maternal genotyping. However, it is clear that the placenta plays a key role in the pathogenesis of this disease, because its expulsion terminates the syndrome. Therefore, the fetal genes may be of critical importance influencing the mother's susceptibility to PE, but at the present little is known about placental COMT genotypes and PE. Additionally, previous data further suggest that COMT activity is more precisely determined by three haplotypes of four SNPs inherited together, rs6269, rs4633, rs4818, and rs4680, which may result in a 25-fold difference in enzyme activity (12). In this regard, Hill et al. (13) reported the association of the low-activity COMT haplotype with an increased risk for preeclampsia in Chilean maternal-fetal dyads population.

Elevated homocysteine has been considered to be a risk factor for vascular dysfunction, vascular disease, and preeclampsia (14, 15). The methionine-homocysteine metabolism is responsible for supplying COMT with the methyl-group donor (*S*-adenosyl methionine; SAM) necessary for 2-ME synthesis, and alterations in this pathway may be related to inability to sustain adequate concentrations of 2-ME during pregnancy, leading to the classic clinical picture of PE (16). Low levels of SAM may be caused by the presence of single-nucleotide polymorphisms (SNPs) in the genes that code for the enzymes involved in SAM metabolism. Methylenetetrahydrofolate reductase (MTHFR) modulates the availability of SAM, and the minor T allele of the MTHFR C677T SNP has been associated with reduced MTHFR activity, resulting in a decrease in SAM production (17). This may be important, because the low-activity COMT and MTHFR genotypes are frequently associated in cases of PE (13).

The purpose of the present study was to investigate the relationship between fetal and maternal COMT Val158Met SNP as well as COMT haplotypes with PE in a southern European population (Murcia, Spain). We evaluated the association of the functional variations linked to the COMT SNPs and haplotype, examining differences in expression and activity of the COMT enzyme and maternal 2-ME levels between preeclamptic and normal pregnancies. Finally, we investigated whether the relationship between COMT and PE was influenced by MTHFR genotypes and homocysteine levels.

MATERIALS AND METHODS

Study Population Sample

This was an observational, analytic, and prospective case-control study. One hundred twenty-five pregnant women (53 preeclamptic and 72 normal control pregnancies) were prospectively included in this study from November 2009 to January 2012 at the Hospital Clínico Universitario Virgen de la Arrixaca (HCUVA) in Murcia, Spain. The population sample size calculation was performed for the genotyped SNPs with the use of a relevant genotype frequency of 24% in whites (from the National Center for Biotechnology Information SNP database), aiming at achieving 80% power to detect an odds ratio difference of 3 for the preeclamptic group. The study was stopped early when statistical significance was reached. PE was defined as blood pressure elevation with proteinuria ($\geq$300 mg per 24-hour period). If 24-hour urine collection was not available, then proteinuria was defined as $\geq$2+ with the use of a dipstick test (18). Blood pressure elevation was defined as an arterial pressure $\geq$140/90 mm Hg on at least two occasions separated by a minimum of 6 hours after the 20th week of gestation in women known to be normotensive before pregnancy as well as during the first weeks of gestation. Pregnant control subjects were women with uncomplicated pregnancies that lasted $\geq$37 weeks (or $\geq$259 days). All patients had singleton pregnancies and white race. None of them had a history of chronic hypertension, renal or infectious diseases, or pregestational or gestational insulin-dependent diabetes. The Ethics Committee of Clinical Investigation of the HCUVA and the Bioethics Committee of the Universidad de Murcia approved the research protocol, including the consent form. Written informed consent was obtained from each participant, and the study was conducted in accordance with the Declaration of Helsinki.