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Association of pregnancy-associated plasma protein A polymorphism with preeclampsia — A pilot study

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ABSTRACT

Objectives: The aim of the study was to investigate genetic and biochemical background of PAPP-A (pregnancy-associated plasma protein A) in patients with risk pregnancies.
Design and methods: Five *PAPP-A* gene polymorphisms and PAPP-A maternal serum levels were studied together in 165 women in third trimester pregnancies complicated with threatening preterm labor ($n=98$), preeclampsia ($n=35$), intrauterine growth restriction ($n=34$) and ICP (intrahepatic cholestasis of pregnancy) ($n=15$). 114 healthy pregnant women served as controls.
Results: Preeclamptic patients had significantly higher frequency of TT genotype of Cys327Cys polymorphism compared to controls ($p<0.01$). Patients with ICP had increased serum levels of PAPP-A compared to controls and correlation analysis showed significant relationship between PAPP-A and CRP (C-reactive protein) in the patients with intrauterine growth restriction ($r=0.49$, $p=0.007$).
Conclusion: Our study indicates the association of TT genotype of Cys327Cys polymorphism of the *PAPP-A* gene with preeclampsia. However, further study with larger groups of preeclamptic patients is needed to confirm our results.

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Introduction

Pregnancy-associated plasma protein A (PAPP-A) is a zinc-binding metalloproteinase, originally found in the plasma of pregnant women in 1974 [1]. Following research revealed positive correlation between PAPP-A serum levels and preeclamptic pregnancies [2]. Klopper [3] showed that the change is most marked in more severe grades of preeclampsia and the higher level of PAPP-A preceded the advent of hypertension and albuminuria. In 1990, Brambati et al. have published, that low maternal serum level of PAPP-A in first trimester of pregnancy is characterized for Down syndrome fetus development [4]. Since then, PAPP-A molecule is considered to be one of the most important biochemical markers of fetal aneuploidies. Its levels can be detected in very low concentrations even in healthy individuals (both non-pregnant women and men). Bayes-Genis et al. [5] hypothesised, that PAPP-A might be a promising marker of acute coronary syndromes. Increased PAPP-A serum levels were detected in patients with renal failure [6] and were found to be related to prognosis of hemodialysis patients [7].

The *PAPP-A* gene is located on chromosome 9q33.1 and contains 22 exons. 200 kb long gene encodes for metalloproteinase of metzincin superfamily [8,9], which specifically cleaves IGF-binding protein (IGFBP) 4, resulting in release of IGF bound to IGFBP-4 [10]. Expression of PAPP-A during pregnancy is located to placental syncytiotrophoblast. PAPP-A is also expressed in ovarian granulosa cells, and in non-reproductive tissues, such as fibroblasts, osteoblasts and vascular smooth muscle cells [11] in concentrations 100–1000 times lower than in placenta.

Preeclampsia, preterm labor, intrauterine growth restriction (IUGR) and intrahepatic cholestasis of pregnancy (ICP) in pregnancy are leading cause of maternal and perinatal morbidity and mortality worldwide, but the underlying pathogenetic mechanisms of these diseases still remain unknown.

Preeclampsia occurs only in pregnant women and is characterized as a development of hypertension after 24th week of pregnancy, in most cases accompanied with proteinuria. Although pathogenesis of the disease is not fully described yet, immune maladaptation, placental ischemia and increased oxidative stress are believed to be of

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etiologic importance. It is likely that preeclampsia is a multifactorial disease, combining both genetic and environmental factors. Research of the genetic background of preeclampsia is mainly focused on candidate genes related to thrombophilia, oxidative stress, lipid metabolism and renin–angiotensin system, including fms-like tyrosine kinase 1 (*Flt-1*) gene [12,13]. Genes like *AGT*, renin, *eNOS* and other were studied and found to be related to essential hypertension in common population as well [14–17].

Preterm labor refers to the birth of a baby of less than 37 weeks gestational age. Infections play a major role in the pathogenesis of preterm labor, and as in the case of preeclampsia, it involves both environmental and genetic factors. Many studies have shown the potential role of the particular genes in the process of normal and pathological labor. Investigators have evaluated two major groups of genes involved in the pathophysiology of the preterm labor: first one includes genes associated with the host response to infection/inflammation, second one includes genes associated with the synthesis and degradation of the extracellular matrix [18].

Intrauterine growth restriction is most often caused by placental dysfunction and manifests as a variable syndrome of suboptimal growth and body disproportions. Together with cholestasis of mother in pregnancy, IUGR syndrome is one of the most serious diseases causing perinatal morbidity and mortality worldwide.

In the present study, our aim was to study selected *PAPP-A* polymorphisms and their relationship to PAPP-A serum levels in patients with risk pregnancies and healthy pregnant women as controls.

Materials and methods

Patients

165 Caucasian women (mean age 30.8 ± 4.7 years) from Czech Republic were included in this single center study. All studied patients were in the third trimester of pregnancy. Ninety-eight women (mean age 30.7 ± 5.1 years) with symptoms of threatening preterm labor (contraction, vaginal dilatation, premature preterm rupture of membranes, bleeding) and thirty-five patients (mean age 31.3 ± 4.1 years) with hypertension in pregnancy or preeclampsia were enrolled in the study. Hypertension in pregnancy was diagnosed in previously normotensive women with two repeated systolic blood pressure of 140 mm Hg or more and diastolic blood pressure measurements of 90 mm Hg or more. Twenty-one women had hypertension and proteinuria more than 300 mg per day. Fourteen women with hypertension had no proteinuria at the time of blood collection, but either they finally developed it during follow up or had increased serum levels of uric acid above 320 μmol/L. Therefore all these patients were evaluated together in preeclampsia group. Thirty-four women (mean age 31.1 ± 4.6 years) had a fetus with intrauterine growth restriction and fifteen women (mean age 31.7 ± 3.4 years) suffered from intrahepatic cholestasis of pregnancy. IUGR was defined as a newborn child that was less than 3rd percentile of birth weight for gestational age. ICP was characterized as an increase of mothers' serum levels of both, AST and ALT enzymes, above 0.72 μkat/L (reference range 0.1–0.72 μkat/L) and 0.78 μkat/L (reference range 0.1–0.78 μkat/L), after exclusion of other causes of elevated liver enzymes (e.g. hepatitis type A, B, C; CMV or EBV virus infection). Patients suffered from pruritus.

114 healthy pregnant women (mean age 30.1 ± 4.3 years) were followed up during prenatal care in the Department of Obstetrics and Gynaecology of the General University Hospital in Prague and studied as controls. The women were in the third trimester of pregnancy at the time of joining the study and all of them finally delivered at term.

The study was performed in accordance with principles of the Declaration of Helsinki and approved by the Institution Ethics Review Board. All patients gave their informed consent prior to entering the study.

Samples

Blood samples were collected via puncture of the cubital vein simultaneously with blood collection for other routine examination. Routine biochemical parameters were determined in fresh samples. For biochemical analysis, blood was collected into tubes without anticoagulant, centrifuged for 10 min at 3000 rpm and serum was frozen at −80 °C until the time of assay.

For DNA analysis, blood was collected into tubes with ethylenediaminetetraacetic acid. Tubes were stored at 4 °C and isolation of DNA was performed by modified salting out procedure according to Miller et al. [19] within a week of collection.

PAPP-A assay

PAPP-A was assessed immunochemically, using the TRACE method (time resolved amplified cryptate emission), based on nonradiating energy transfer. Commercial kit KRYPTOR-PAPP-A (Brahms, Germany) was used for determination of the PAPP-A level in the maternal serum. The results are expressed in IU/L.

Routine laboratory parameters

Routine biochemical parameters were assessed by commercially available kits using certified biochemical techniques. C-reactive protein (CRP) was determined turbidimetrically (Roche Diagnostics, Modular analyzer, Roche Diagnostics GmbH, Germany). Serum creatinine was determined using Jaffe's method (Modular analyzer, Roche Diagnostics GmbH, Germany). Total protein measurement on the 24-h urine sample was performed on the Roche Modular System (benzethonium chloride) on the same day as collections were completed. Serum ALT and AST levels were determined with the IFCC modified method at 37 °C with addition of the pyridoxal-5-phosphate (Modular analyzer, Roche Diagnostics GmbH, Germany). Uric acid was evaluated enzymatically (Modular analyzer, Roche Diagnostics GmbH, Germany). Urea serum levels were determined by enzymatic UV assay with urease (Modular analyzer, Roche Diagnostics GmbH, Germany) and total bilirubin was assessed by the method with stabilized diazonium salt (Modular analyzer, Roche Diagnostics GmbH, Germany).

Blood count was measured with an automated hematological Beckman Coulter LH750 Hematology analyzer (Beckman Coulter, USA).

PAPP-A gene analysis

Two DNA fragments containing exon 2 (323 bp) and exon 6 with the part of intron 6 (387 bp) of the *PAPP-A* gene were amplified by polymerase chain reaction (PCR) with primers summarized in Table 1. The PCR proceeded with initial denature at 94 °C for 2 min, followed by 33 cycles of 94 °C for 30 s, 60 °C for 45 s and 72 °C for 1 min, and additionally 72 °C for 7 min. The primers were predicted by Primer3 Input web application (http://frodo.wi.mit.edu/).

Products were separated by electrophoresis in 2% agarose gel, excised from the gel and purified with spin columns (NucleoSpin

Table 1
Primers and PCR conditions.

Exon	Primer sequence 5′→3′	Annealing T (°C)	Product size (bp)
2	Forward: gga ctc agc ggg aga tac tg Reverse: cac cgt cgg gtt ctt atg at	60.0	323
6	Forward: cta gtc cca tca gtt gct cat tc Reverse: tgt tag gat agt gca agt gct ca	60.0	387

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