Short Communication

Pregnancy-Associated Plasma Protein A Polymorphisms in Patients with Risk Pregnancies

(pregnancy-associated plasma protein A / PAPP-A / pregnancy / preeclampsia / preterm labour / single nucleotide polymorphism / SNP / gene sequencing)

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Abstract. Pregnant women are often threatened by hypertension, symptoms of preterm labour, hepatopathy, and other. These complications might be the consequence of genetic factors together with involvement of environmental factors. We were searching for three polymorphisms Arg654Lys, Ala678Pro and Thr686Ala in exon 5, and two polymorphisms Phe802Leu, Ser827Ser/Leu in exon 7, and for the new mutations in exons 5 and 7 of the pregnancy-associated plasma protein A gene in the studied group consisting of 203 women – 79 pregnant women in time of preterm labour, 24 pregnant women suffering from preeclampsia, and 100 healthy pregnant and non-pregnant women serving as controls. We did not find any divergence from wild-type form of these polymorphisms in any of the studied groups, which led us to the hypothesis that these polymorphisms are not associated with our studied group of Caucasian origin. However, further studies with a larger group of subjects are needed to confirm our results.

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Abbreviations: AGT – angiotensinogen, AMI – acute myocardial infarction, eNOS – endothelial nitric oxide synthase, IGFBP – insulin-like growth factor-binding protein, NCBI – The National Center for Biotechnology Information, PAPP-A – pregnancy-associated plasma protein A, PCR – polymerase chain reaction, proMBP – proeosinophil major basic protein, RPL – recurrent pregnancy loss.

Introduction

Pregnancy-associated plasma protein A (PAPP-A) is a homotetrameric glycoprotein originally found by Lin et al. (1974) in the plasma of pregnant women. PAPP-A exists in pregnancy serum as a heterotetrameric 2 : 2 complex with the proform of eosinophil major basic protein (proMBP), called PAPP-A/proMBP complex (Oxvig et al., 1993). This insulin-like growth factor-binding protein (IGFBP) proteinase (Lawrence et al., 1999) belongs to the metzincin superfamily of metalloproteinases (Bode et al., 1993) and is mainly produced by the placental syncytiotrophoblast during pregnancy. PAPP-A is also expressed in ovarian granulosa cells, and in non-reproductive tissues, such as fibroblasts, osteoblasts and vascular smooth muscle cells (Overgaard et al., 1999).

PAPP-A is one of the most important biochemical markers of foetal aneuplodies in pregnancies. Its low maternal serum level in the first trimester of pregnancy is characteristic for Down syndrome foetus development (Brambati et al., 1990). On the contrary, PAPP-A serum levels are significantly increased in preeclamptic pregnancies (Lin et al., 1977). Klopper (1982) 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. Moreover, Bayes-Genis et al. (2001) hypothesed that PAPP-A might be a promising marker of acute coronary syndromes.

The human *PAPPA* gene is located on chromosome 9q33.1, spans over 200 kb of DNA, and contains 22 exons (Overgaard et al., 2003).

In the present study, we analysed five *PAPPA* polymorphisms (Arg654Lys, Ala678Pro, Thr686Ala, Phe802Leu, Ser827Ser/Leu) and searched for the new mutations in exons 5 and 7 of the *PAPPA* gene in the pregnancies complicated with preeclampsia or preterm labour, and in healthy pregnant and non-pregnant women as controls to improve our understanding of the genetic background of these complications in pregnancy.

Material and Methods

Patients

The studied group consisted of 203 women – 79 pregnant women in time of preterm labour, 24 pregnant women suffering from preeclampsia, and 100 healthy pregnant and non-pregnant women.

One hundred and three Caucasian women (mean age 30.1 ± 4.8 years) from the Czech Republic were included in this single-centre study. Seventy-nine women with symptoms of threatening preterm labour (contraction, vaginal dilatation, premature preterm rupture of membranes, bleeding) and 24 patients with hypertension in pregnancy were enrolled into the study. Hypertension in pregnancy was diagnosed in previously normotensive women with two repeated diastolic blood pressure measurements of 90 mm Hg or more and systolic blood pressure of 140 mm Hg or more. Eleven women had proteinuria higher than 300 mg per day; 13 women did not suffer from proteinuria. The women were between 24th and 40th week of pregnancy.

Fifty-two healthy pregnant women (mean age 29.4 ± 4.6 years) were followed up during prenatal care at the Department of Gynaecology and Obstetrics of the General University Hospital in Prague and studied as controls. The women were between 8th and 41st week of pregnancy.

Forty-eight healthy non-pregnant women served as controls. Mean age of non-pregnant controls was 26.1 ± 4.2 years.

The study was performed in accordance with principles of the Declaration of Helsinki and approved by the Institutional Ethical Committee. All patients have given their informed consent prior to entering the study.

Samples

Blood of the patients was collected via puncture of the cubital vein simultaneously with blood collection for other routine examinations. Tubes with ethylene diamine tetraacetic acid were used for DNA analysis. Blood samples were stored at 4 °C and isolation of DNA was performed by the modified salting-out procedure according to Miller et al. (1998) within a week.

PAPPA gene sequencing

Both exons 5 and 7 of the *PAPPA* gene were amplified by polymerase chain reaction (PCR) with primers and annealing temperatures summarized in Table 1. Products were separated by electrophoresis in 2% agarose gel, excised from the gel and purified with spin columns (NucleoSpin Extract II, Macherey-Nagel, Düren, Germany). Purified DNA was then sequenced in genetic analysis system CEQ 8000 (Beckman Coulter, Brea, CA) according to the manufacturer's protocol.

The primers were predicted by Primer3 Input (http://frodo.wi.mit.edu).

Results and Discussion

We were searching for three polymorphisms Arg654Lys (dbSNP: rs432500), Ala678Pro (dbSNP: rs34371232) and Thr686Ala (dbSNP: rs35578777) in exon 5, and two polymorphisms Phe802Leu (dbSNP: rs1063409), Ser827Ser/Leu (dbSNP: rs34087604) in exon 7, but we did not find any divergence from the wild-type form of these polymorphisms in any patients or controls. Consequently, we did not find any difference in the subgroup of patients with preeclampsia and preterm labour as well as in the subgroup of pregnant and non-pregnant controls (Tables 2, 3).

Beside that, we were looking for new mutations in exons 5 and 7 of the *PAPPA* gene, but again, we did not find any divergence from reference sequences in any of the patients or controls.

In this study, we for the first time analysed overall five polymorphisms localized in exons 5 and 7 of the *PAPPA* gene.

SNPs in these areas are described in The National Center for Biotechnology Information (NCBI) variation database (dbSNP); however, no study focused on *PAPPA*

Table 1. Primers and PCR conditions

Exon	Primer sequence 5′ → 3′	Annealing T (°C)	Product size (bp)
5	F: cgg ctt ggt gct tat ctc tc R: aca ggg cac act cac ctt tc	60.0	245
7	F: gct ctt tcc cca aga act ca R: acc tca cgt tcc tcc aca ac	59.7	602

F – forward, R – reverse, T – temperature

Table 2. Alleles of the studied PAPPA gene polymorphisms and their frequencies in our studied group

Exon	SNP	Wild-type allele (frequency)	Mutant allele (frequency)
5	*Arg654Lys*	G (1.00)	A (0.00)
	Ala678Pro	G (1.00)	C (0.00)
	Thr686Ala	A (1.00)	G (0.00)
7	*Phe802Leu*	T (1.00)	C (0.00)
	Ser827Ser/Leu	TC (1.00)	C-/-- (0.00/0.00)

Table 3. Genotype distribution in patients and healthy controls (number of patients)

SNP	**Patients**		**Controls**	
Genotypes	**Preeclampsia**	**Preterm labour**	**Pregnant**	**Non-pregnant**
Arg654Lys				
GG	24	79	52	48
GA	0	0	0	0
AA	0	0	0	0
Ala678Pro				
GG	24	79	52	48
GC	0	0	0	0
CC	0	0	0	0
Thr686Ala				
AA	24	79	52	48
AG	0	0	0	0
GG	0	0	0	0
Phe802Leu				
TT	24	79	52	48
TC	0	0	0	0
CC	0	0	0	0
Ser827Ser/Leu				
TC	24	79	52	48
C-	0	0	0	0
- -	0	0	0	0

polymorphisms located both in exon 5 – Arg654Lys, Ala678Pro, Thr686Ala, and exon 7 – Phe802Leu, Ser827Ser/Leu has been published to date.

Both preeclampsia and preterm labour are conditions influenced by multiple genes together with involvement of environmental factors. In our study, we tried to find any association between polymorphisms in exons 5 and 7 of the *PAPPA* gene and these complications in pregnant women. We were searching for Arg654Lys, Ala678Pro, Thr686Ala, Phe802Leu and Ser827Ser/Leu polymorphisms of the *PAPPA* gene, but we did not find any divergence from the wild-type form of these polymorphisms either in patients or in healthy subjects serving as controls. Consequently, we did not find any difference in the subgroup of patients with preeclampsia and preterm labour as well as in the subgroup of pregnant and non-pregnant controls.

Studies of more than 50 candidate genes have reported their potential role in pathophysiology of preeclampsia, including several genes involved in the blood pressure regulation and vascular remodelling (Mütze et al., 2008). Genes like *AGT*, rennin, *eNOS*, and other were studied and found to be related to essential hypertension in common population as well (Jeunemaitre et al., 1992; Jáchymová et al., 2001; Hasimu et al., 2003).

Similarly, many studies have shown the potential role of the particular genes in the process of normal and pathological labour. These genes fall into two major groups including those involved in the host response to infection/inflammation, and those involved in the synthesis and degradation of the extracellular matrix (Anum et al., 2009). As yet, no study focused on *PAPPA* polymorphisms in patients with preeclampsia or preterm labour has been published.

Only two studies have focused on the polymorphisms of the *PAPPA* gene so far. Suzuki et al. (2006) investigated whether the maternal *PAPPA* polymorphism Ser1224Tyr (dbSNP: rs7020782, exon 14) could be associated with the risk of recurrent pregnancy loss (RPL). He found out that women carrying the C allele have an increased risk of RPL with at least one pregnancy loss after 9-week gestation. All patients and the controls involved in the study were native Japanese women.

Another study showed association of *PAPPA* polymorphism with an increased risk of acute myocardial infarction (AMI) in Korean population. Park et al. (2007) analysed four *PAPPA* polymorphisms and seven other polymorphisms of cytokine genes that have been reported to have functional significance, and they defined C allele of the *PAPPA* SNP rs13290387 (6.intron) as an independent risk factor for AMI. Furthermore, they did not find any significant association between AMI and *PAPPA* polymorphism Ser1224Tyr.

Although biochemical studies have brought many important findings of clinical aspects of the pregnancy-associated plasma protein A not only in prenatal screening of Down syndrome, but also in patients with acute coronary syndrome or renal diseases (Lin et al., 1977; Brambati et al., 1990; Bayes-Genis et al., 2001; Kalousová et al., 2004), focus on the genetic background of the *PAPPA* gene could be a new promising trend, helpful in better understanding of the pathological mechanisms in which PAPP-A is involved.

In summary, in this study we were searching for overall five polymorphisms localized in exons 5 and 7 of the *PAPPA* gene and for new mutations. We did not find any divergence from the wild-type form of these polymorphisms in any of the studied groups, which led us to the

hypothesis that these polymorphisms are not associated with our studied group of Caucasian origin. However, further studies with a larger group of subjects are needed to confirm our results.

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None of the authors declared conflict of interest.

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