Placental protein 13 (PP13): a new biological target shifting individualized risk assessment to personalized drug design combating pre-eclampsia

Berthold Huppertz1,*, Hamutal Meiri2, Sveinbjorn Gizurarson3, George Osol4, and Marei Sammar5

1Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Harrachgasse 21/7, Graz 8010, Austria 2TeleMarpe Ltd., 12 Barasani St., Suite 48, Tel Aviv, Israel 3Faculty of Pharmaceutical Sciences, School of Health Science, University of Iceland, Reykjavik, Iceland 4Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont College of Medicine, Burlington, VT, USA 5Department of Biotechnology Engineering, ORT Braude College, Karmiel, Israel

*Correspondence address. Tel: +43-316-380-7604; E-mail: berthold.huppertz@medunigraz.at

Submitted on November 27, 2012; resubmitted on January 10, 2013; accepted on January 16, 2013

TABLE OF CONTENTS

- Pre-eclampsia
  - The features of the disorder
  - Predictive markers for pre-eclampsia
- Placental protein 13 (PP13)
  - PP13 is galectin 13
  - PP13 mRNA in healthy and preeclamptic tissues
  - The LGALS13 gene in the placenta and its polymorphisms
  - Functional characterization of PP13
  - Longitudinal assessment of PP13 in vivo and in vitro
  - Pre-eclampsia prophylaxis
- Predicting the risk for pre-eclampsia with PP13: a meta-analysis
  - Immunoassays
  - Methods and included studies for the PP13 meta-analysis
  - Forest plot results of PP13 meta-analysis
  - Multiple markers
- From individualized risk to drug development
  - The concept
  - Hypothesis: Keeping PP13 levels within a ‘therapeutic window’ is essential to prevent pre-eclampsia
  - Pilot study
  - Could PP13 aid in preventing pre-eclampsia?

BACKGROUND: Pre-eclampsia affects 2–7% of all pregnant women and is a major cause of maternal and fetal morbidity and mortality. The etiology of pre-eclampsia is still unknown but it is well documented that impaired placentation is a major contributor to its development. One of the placenta-specific proteins is placental protein 13 (PP13). Lower first trimester levels of maternal serum PP13 and its encoding placental mRNA are associated with the development of both early and late-onset severe pre-eclampsia. In cases where this protein is mutated, the frequency of pre-eclampsia is higher.
Pre-eclampsia

The features of the disorder

Definition of pre-eclampsia

Pre-eclampsia is a life-threatening condition without a clearly understood etiology. It is defined by the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988; Brown et al., 2001; Lindheimer et al., 2009) as the development of new onset hypertension of 140/90 mmHg (systolic/diastolic) or greater after 20 weeks of gestation in previously normotensive women coupled to elevated protein levels in urine (>300 mg/dl). Hypertension and/or proteinuria may become severe, reaching levels >160/110 mmHg and >3 g/dl protein (‘severe pre-eclampsia’). It may further progress to eclampsia, an obstetric emergency associated with brain convulsion, cerebral edema and stroke, a life-threatening condition. It is defined by the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988; Roberts and Cooper, 2001). The mortality ratio to die due to pre-eclampsia is increased 3.3-fold compared with term controls when the disorder occurred at 33–36 weeks of gestational age (GA), and even 12.5-fold when pre-eclampsia occurred at 28 or less weeks of GA (MacKay et al., 2001). According to the National Institute of Child Health and Human Development (NICHD), 25–27% of women with pre-eclampsia require preterm delivery, and the earlier the delivery occurs the more severe the complications are for both mother and baby (Myatt and Miodovnik, 1999). More than 50% of early pre-eclampsia cases are delivered by Cesarean section as compared with 50% in pre-eclampsia at term and 15–18% in the entire population (Douglas and Redman, 1994). Moreover, pre-eclampsia is associated with an increased frequency of obesity, diabetes and cardiovascular disorders in affected women later in life (Smith et al., 2001).

Early pre-eclampsia cases account for only 0.4–1.4% of the entire pregnancy population; however, they have a much higher frequency of eclampsia, cerebro-vascular accidents and other severe complications compared with pre-eclampsia at term (Douglas and Redman, 1994). There is an annual life loss of ≦500 000 newborns due to pre-eclampsia, particularly in early cases, the latter being of high risk to be growth restricted and becoming severely handicapped, developing cerebral palsy or even dying in utero. Zwart et al. (2008) have found that 33.2% of severe pre-eclampsia cases [including early and intermediate cases (28.8%) and late cases (71.2%)] were admitted to intensive care units (ICU) or coronary care in addition to the standard post-operative recovery, 7.5% had uterine rupture and underwent hysterectomy or laparotomy, 5.3% developed eclampsia or severe HELLP syndrome, 44.9% needed transfusion and 9.1% delivered prematurely and their newborns suffered from severe motor and cognitive disorders or blindness.

Epidemiology of pre-eclampsia

Pre-eclampsia affects 2–7% of all pregnancies and still is a major cause of maternal death accounting for 18% of all women’s deaths in pregnancy (Davey and MacGillivray, 1988; Roberts and Cooper, 2001). The incidence of severe pre-eclampsia is higher in developing countries as compared with 33.2% in the United States of America (US). The risk to die due to pre-eclampsia is increased 3.3-fold compared with term controls when the disorder occurred at 33–36 weeks of gestational age (GA), and even 12.5-fold when pre-eclampsia occurred at 28 or less weeks of GA (MacKay et al., 2001). According to the National Institute of Child Health and Human Development (NICHD), 25–27% of women with pre-eclampsia require preterm delivery, and the earlier the delivery occurs the more severe the complications are for both mother and baby (Myatt and Miodovnik, 1999). More than 50% of early pre-eclampsia cases are delivered by Cesarean section as compared with 50% in pre-eclampsia at term and 15–18% in the entire population (Douglas and Redman, 1994). Moreover, pre-eclampsia is associated with an increased frequency of obesity, diabetes and cardiovascular disorders in affected women later in life (Smith et al., 2001).

Early pre-eclampsia cases account for only 0.4–1.4% of the entire pregnancy population; however, they have a much higher frequency of eclampsia, cerebro-vascular accidents and other severe complications compared with pre-eclampsia at term (Douglas and Redman, 1994). There is an annual life loss of ≦500 000 newborns due to pre-eclampsia, particularly in early cases, the latter being of high risk to be growth restricted and becoming severely handicapped, developing cerebral palsy or even dying in utero. Zwart et al. (2008) have found that 33.2% of severe pre-eclampsia cases [including early and intermediate cases (28.8%) and late cases (71.2%)] were admitted to intensive care units (ICU) or coronary care in addition to the standard post-operative recovery, 7.5% had uterine rupture and underwent hysterectomy or laparotomy, 5.3% developed eclampsia or severe HELLP syndrome, 44.9% needed transfusion and 9.1% delivered prematurely and their newborns suffered from severe motor and cognitive disorders or blindness.
normal term controls and term pre-eclampsia, while it was reduced to 73% in intermediate pre-eclampsia and to 41% in early pre-eclampsia. Total placental volume, intervillous space volume and volume of terminal villi were all significantly lower in early pre-eclampsia compared with normal outcome, whereas pre-eclampsia at term had no impact on peripheral villous volume or vascular features and were morphologically similar to placentas from gestational age-matched healthy controls (Egbor et al., 2006).

Reduced placental size in pre-eclampsia is accompanied by increased release of cell-free fetal DNA and RNA into maternal blood (particularly in early pre-eclampsia) compared with normal pregnancies (Zhong et al., 2006). While shedding of apoptotic corpuscular structures (syncytiotrophoblast) is part of the normal turnover of villous trophoblast throughout pregnancy (Huppertz, 2008), in pre-eclampsia this process is dysregulated by the release of subcellular, necrotic microparticles (STBM) particularly in the second and third trimester (Goswami et al., 2006). Increased shedding of necrotic material provokes the systemic inflammatory response and endothelial damage of the mother. Goswami et al. (2006) identified significantly elevated levels of STBM in maternal blood in preeclampsics compared with controls for term and early pre-eclampsia, but found no changes in IUGR and preterm delivery not complicated by pre-eclampsia (Goswami et al., 2006). The presence of higher amounts of trophoblast fragments and proteins, microvillous membranes and cell-free fetal DNA in maternal blood of preeclamptic women points to the fact that the placenta is the likely inducer of the maternal inflammatory response (von Dadelszen et al., 2003).

**Long-term effects of pre-eclampsia**

In the majority of cases, pre-eclampsia develops at term or immediately after delivery, and mainly affects maternal organs. However, the stressful experience in the womb also has a major impact on the epigenetics of the fetus (Choudhury and Friedman, 2012). Not only the baby, but also the mother may experience long-term consequences due to pre-eclampsia. Smith et al. (2001) analyzed maternity discharge data of 129,920 singleton deliveries of live babies and matched this information with the Scottish Morbidity Record system and general registrar’s office death database. They reported a significantly increased rate of ischemic heart disease, including death, 15–19 years after these women experienced early pre-eclampsia in their first pregnancy (Smith et al., 2001).

Mongraw-Chaffin et al. (2010) analyzed the risk of cardiovascular disease death in 14,403 women with and without pre-eclampsia in their pregnancies using a median follow-up time of 37 years. At 30 years of follow up, in the group of women experiencing early pre-eclampsia only 85.9% survived, while in the group of women with late pre-eclampsia 98.3% and in the group without pre-eclampsia 99.3% survived. This is a clear demonstration that women experiencing pre-eclampsia have an increased risk of cardiovascular disease death later in life, independent of traditional risk factors.

Irgens et al. (2001) showed the long-term effect of pre-eclampsia after they had examined data from 626,272 women. They found that preterm delivery and, to a higher extent, early pre-eclampsia was associated with a significant shortening of maternal longevity by ~13 years (on average) after first delivery. According to this study, the death ratio was 15.1 per 1000 for early pre-eclampsia, 8.3 for intermediate pre-eclampsia and 6.6 per 1000 for either term pre-eclampsia or term non-preeclamptic women. Furthermore, these and other authors (Fraser et al., 2012) have also found an increased risk for cardiovascular diseases among women who experienced pre-eclampsia during their pregnancies, particularly intermediate and early pre-eclampsia. The hazard to death ratio from cardiovascular disease was 8.1 times higher in early pre-eclampsia and 2.7 in term pre-eclampsia. The elevated risk for death from stroke was 5.1 times higher in early pre-eclampsia and 1.6 for term pre-eclampsia compared with normal outcome.

**Prediction, surveillance and conservative management**

The standard practice in obstetrics/perinatology is to evaluate a woman’s risk for pre-eclampsia based on previous medical, obstetrical and demographic data. The prediction accuracy following this practice provides at best 30–40% detection rate (DR) for a 10% false-positive rate (FPR). The frequency of pre-eclampsia is 19% after a previous pre-eclampsia, 25 and 22% for chronic hypertension and diabetes and 18% in nephropathy, all accounting to the highest risk of pre-eclampsia (Cartis et al., 1998; Myatt and Miodovnik, 1999).

According to Sibai et al. (Sibai et al., 1994; Sibai, 2011) women identified earlier in pregnancy could be followed by conservative methods which may improve maternal and baby outcomes. These authors have shown that gestational age at delivery is the most crucial determinant for newborn outcome that can be improved by conservative management. If gestational age at delivery is increased from 31 to 33 weeks GA, the result will be a higher birthweight, a lower incidence of admission to the neonatal ICU (76 versus 100%), lower mean days of hospitalization in the ICU (20 versus. 37) and a lower incidence of neonatal complications. The studies imply that it is valuable to identify women at risk for pre-eclampsia very early in pregnancy to optimize successful conservative management. Moreover, even in the absence of a verified treatment for pre-eclampsia, the American College of Obstetricians and Gynecologists as well as the Royal College of Obstetricians and Gynecologists recommend increased surveillance in at-risk groups to provide better care (ACOG Practice Bulletin, 2002).

A study in the healthcare service of MaternaLink (now Alere) reviewed its Disease Management Program published in December 2001 (MaternaLink, 2001) that involved education of women to self-awareness and life style changes and close surveillance. The study demonstrated a cost reduction of nearly $45 million for 120,000 births over a 4 year period due to close surveillance coupled with patient awareness. Neonatal ICU (NICU) days per 1000 births in the group who participated in the education and awareness group dropped from 1194 in 1997 to 1013 in 2000, significantly below the USA national average of 1200–1500 NICU days per 1000 births. This was even more dramatic for patients who participated in the program for 3 years, plummeting to 970 days per 1000 births in 2000. Additional benefits according to this report included the following:

(i) Delivery before 34 weeks for the study participants due to early pre-eclampsia dropped to 0.6% of total deliveries compared with the national average of 2.0%.
(ii) Delivery before 37 weeks due to preterm pre-eclampsia was 0.9% of total deliveries compared with the national benchmark of 2.3%.
(iii) Low birthweight due to pre-eclampsia was 1.3% compared with the national average of 2.9%.
According to the NICHD (Myatt and Miodovnik, 1999) women suffering from early pre-eclampsia belong to the group that is in greatest need of early detection for its life saving and premature prevention. Meta-analyses have shown that treating women at risk for pre-eclampsia by low-dose aspirin could reduce the frequency of pre-eclampsia, provided that the treatment starts before 16 weeks of gestation (Bujold et al., 2009, 2010). Accordingly, the World Health Organization (WHO, 2011) and the National Institute for Clinical Excellence (NICE) of the UK issued guidelines that recommend treatment with aspirin if the patients are at risk for pre-eclampsia (NICE guidelines, 2010) starting from before 16 weeks of gestation (according to Bujold et al., 2010) and continuing to near delivery.

**Predictive markers for pre-eclampsia**

**WHO requirements and relevance of markers**

Requirements: the criteria of the WHO for a satisfactory screening test include the following requirements:

- a well-defined disorder,
- a known prevalence,
- a disease where the management can improve outcome and decrease severity, and
- the creation of a cost/effective rationalization (Conde-Agudelo et al., 2004)

As a secondary requirement, the WHO lists the ease of installing the screening test, its simplicity and ease of performance and its safety to the patients and performers. The conceived importance by patients and professionals, the development of suitable cutoff levels to separate the patients and performers. The conceived importance by patients screening test, its simplicity and ease of performance and its safety to

Relevance: the development of biomarkers to predict pre-eclampsia was started with markers that can predict the disorder 2–4 weeks before the onset of clinical symptoms (Levine et al., 2004). The major success in this direction was achieved using the increase in serum soluble fms-like tyrosine kinase-1 (sFlt-1) combined with the decrease in serum placental growth factor (PIGF). The increased sFlt-1:PIGF ratio was developed as the major tool to identify the risk to develop pre-eclampsia near the time of onset of clinical symptoms (Levine et al., 2004). Today there are other second trimester effective markers of pre-eclampsia, particularly activin-A and inhibin-A (Spencer et al., 2006).

However, since early prediction is preferred, the most accurate prediction was developed combining various markers tested in first trimester:

(i) The importance of the placenta becomes obvious by the capability of first trimester low protein markers derived from the placenta including PIGF, plasma-associated placental protein A (PAPP-A) and placental protein 13 (PP13) in predicting early pre-eclampsia, especially when complicated with IUGR (Akolekar et al., 2009).

(ii) Elevated sFlt-1, soluble endoglin, TGF-beta and reduced vascular endothelial growth factor are strong markers of endothelial inflammation and the respective maternal complications (Romero et al., 2008a).

(iii) Lifelong complications are associated with changes in the coagulation system, the complement system, C reactive protein (CRP) and other metabolic markers, all used in monitoring later life risks for cardiovascular disorders and diabetes (Mihu et al., 2008).

(iv) Consequences to the fetus are best addressed through the use of ultrasound including uterine arteries and umbilical cord and other fetal arteries quantifying the pulsatility index measured by Doppler ultrasound (Harrington et al., 1997).

**Ultrasound and biochemical markers**

**Ultrasound:** abnormal second trimester uterine artery Doppler ultrasound is capable of identifying women at risk of early pre-eclampsia (Papageorghiou et al., 2002). Elaboration of uterine artery Doppler ultrasound has also indicated its effectiveness in early pre-eclampsia prediction in the first trimester (Martin et al., 2001). Nowadays, almost all scan machines have incorporated Doppler ultrasound and with training workshops offered by the fetal medicine foundation and others, sonographers can perform adequate measurements. The sensitivity and specificity of the elevated pulsatility index of uterine artery Doppler ultrasound (Doppler PI) in the first trimester for early onset pre-eclampsia are around 0.65 and 0.9, respectively. Accordingly, some medical centers use elevated Doppler PI values as a measure for preventive interventions such as aspirin in addition to the recommendations of the NICE guidelines. Doppler PI, however, is limited for predicting early and intermediate pre-eclampsia and has a much lower performance for late onset pre-eclampsia.

**Biochemical markers:** There is a long list of hormones, e.g. beta-hCG, human placental lactogen (hPL), estrogen and progesterone and protein biomarkers (PAPP-A, PIGF, sFlt-1, activin-A, inhibin-A, sEndoglin, etc.) that are important for the development and function of placenta and pregnancy. Many of them were proved to be effective in predicting pre-eclampsia. So far, the most effective first trimester biomarkers are PP13 and PIGF, both placentally derived proteins that have been detected in maternal serum already at 5–6 weeks of gestation. This review is focused on PP13.

**Placental protein 13**

**PP13 is galectin 13**

Initially isolated by Bohn et al. (1983), PP13 is one of the 56 known placental proteins identified so far. PP13 is a 32-kDa homo-dimer protein and was purified from term placenta (Bohn et al., 1983). Its full length sequence shows an open-reading frame of 354 base pairs encoding a 118 amino acid polypeptide (Than et al., 1999; Burger et al., 2004).

Sequence analysis revealed that PP13 is a member of the galectin super-family (defined as galectin 13), a family of carbohydrate-binding proteins called β-galactoside-specific lectins. They are defined by their b-galactoside-specific lectins. They are defined by their
The gene locus for PP13, LGALS13, is on chromosome 19 near five other members of the galectin family (Than et al., 2009). The clustering has emerged during primate evolution and it was proposed that this is a result of duplication and rearrangement of genes and pseudogenes via a birth and death process of transposable long interspersed nuclear elements. The clustering is particularly abundant among primates with a large brain and long gestation. LGALS13 and also LGALS14 and LGALS16 are placenta specific and hardly, if any, expression can be found in other tissues (Than et al., 2009).

Intrigued by the finding of lower PP13 mRNA in pre-eclampsia, the group of Hillerman examined different ethnic groups by sequencing the LGALS13 gene in search for polymorphisms relevant to reduced PP13 mRNA (Stolk et al., 2006). These authors have shown that three motives in the LGALS13 gene may lead to polymorphisms: (i) truncation that results in missing exons often brought about by single nucleotide deletion and the introduction of one or more premature stop codons; (ii) mutations of the exon-intron boundaries that lead to differential RNA splicing and (iii) promoter changes that regulate expression levels (Fig. 1A).

LGALS13: delT221 mutation
A deletion of the T nucleotide in position 221 (delT221 mutation) in exon 3 (Fig. 1A) was anticipated to yield a frame shift in the open-reading frame that could lead to a stop codon. The mutation is predicted to translate into a shorter mRNA and respective protein lacking the entire exon 4 and part of exon 3, corresponding to the loss of the major CRD. Sammar et al. (2006) cloned the cDNA of the delT221 mutation and obtained a much shorter protein that is routed to enclusion bodies when expressed in bacterial hosts. Such routing is typical for proteins with impaired folding, and a recovery plan was experimentally implemented to rescue the misfolded protein to partially refold and purify it (Fig. 1B) (Sammar et al., 2006). In humans the mutated protein is instantaneously degraded within the placenta and is not released to maternal blood.

An analysis of the delT221 mutation in pregnant women and their offspring in patients with pre-eclampsia compared with controls was performed among black African women (Bosman et al., 2008) and among low-risk patients of caucasian and colored origin (Gebhardt et al., 2009). When the delT221 mutation appears in the mother as a heterozygous mutation, the positive predictive value to develop very early and severe pre-eclampsia necessitating delivery before 34 weeks was 89% (Sammar et al., 2006; Bosman et al., 2008; Rebello et al., 2009). No vital delT221 homozygous mutation was identified in any of the 1000 patients examined (Bosman et al., 2008). Than et al. (2009) have shown that after PP13 truncation (as caused by the delT221 mutation) the protein loses its ability to induce T-cell apoptosis. Accordingly, it is reasonable to speculate that failure to induce T-cell apoptosis may lead to a higher frequency of pre-eclampsia as was found in heterozygous carriers of delT221. The mutated protein is routed to fast degradation, and is degraded almost instantaneously. The PP13 blood level of heterozygous carriers is low since the unaffected allele produces a lower level of PP13 compared with the two alleles of the wild type.

LGALS13 – Dex-2 mutation
The Dex-2 mutation is a splice variant of PP13 lacking the majority of exon 2 and a few amino acids of exon 3 (Fig. 1B). It was initially isolated from a genomic DNA library of human placenta (Burger et al., 2004) and is derived of intronic G-to-A conversions between exons 2 and 3 (Stolk et al., 2006, Fig. 1A). The splice variant was cloned and expressed and

**PP13 mRNA in healthy and preeclamptic tissues**
Than et al. (2009) have shown the expression of PP13 mRNA in fetal membranes, especially the amnion. In situ hybridization of PP13 mRNA in placental tissues showed specific localization to the syncytiotrophoblast layer (Than et al., 2009).

PP13 mRNA expression profiles show that PP13 is specifically expressed in the placenta compared with other tissues, where only very low if any expression can be identified. This lack of PP13 is true for a very large selection of diversified human tissues from adult and fetal origin (Than et al., 2009).

In studies of placentas obtained after delivery, Than et al. (2008a) and Sammar et al. (2011) found 3.5-fold reduced PP13 mRNA levels in patients with pre-eclampsia and HELLP syndrome compared with controls. The effect was already present in the first trimester. Using laser microdissection, placental villi from chorionic villus sampling obtained from patients who subsequently developed preeclampsia were found to have lower PP13 mRNA compared with healthy cases (Sekizawa et al., 2009). Reduced expression of PP13 mRNA is considered as one of the earliest indications to develop pre-eclampsia. These findings indicate that reduced PP13 mRNA expression in villous trophoblast throughout pregnancy is associated with the pathogenesis of pre-eclampsia. However, the levels of sensitivity and specificity found in this study were low, and thus between 30 and 50% of women with low levels of PP13 mRNA will not develop pre-eclampsia.

In a case–controlled study Shimizu et al. 2009 found that in peripheral blood samples obtained from pregnant women with pre-eclampsia and controls PP13 mRNA blood levels in pre-eclampsia cases were significantly lower than in controls. In a different cohort study of asymptomatic pregnant women, Farina et al. (2010) found that PP13 mRNA from women who later developed pre-eclampsia was already lower in the first trimester compared with controls providing 31% DR at an FPR of 5%. These authors analyzed the concentration of PP13 mRNA in the cellular compartment of maternal blood and found reduced levels of PP13 mRNA in patients in the first trimester who subsequently developed pre-eclampsia. These findings indicate that alterations in PP13 in pre-eclampsia can be assessed indicating a pathophysiological change of PP13 very early in pregnancy. It remains to be seen if the reduced level of PP13 mRNA early in pregnancy is a primary cause or a consequence of impaired placentation.

**The LGALS13 gene in the placenta and its polymorphisms**
The gene locus for PP13, LGALS13, is on chromosome 19 near five other members of the galectin family (Than et al., 2009).
due to its impaired configuration was also routed to enclusion bodies (Sammar et al., 2006). Dex-2 proximity to the N-terminus interferes with PP13 folding to form a dimer by S–S bridges, and generates an unstable PP13. The spliced variant is also degraded within the placenta almost instantaneously and does not reach maternal blood. Intron polymorphisms were quite frequently found among Caucasian African women in association with term pre-eclampsia and lower PP13 mRNA (Bosman et al., 2008; Than et al., 2008b; Gebhardt et al., 2009). Burger et al. (2004) have found that this variant is impaired of its lysophospholipase activity, as will be discussed later.

**LGAL13S: 98A to C replacement polymorphism**

The 98A-C is a promoter single nucleotide replacement (Fig. 1A) found in women with increased risk to develop pre-eclampsia. Bruiners et al. (2007) assessed the risk of developing pre-eclampsia when having this A–C replacement genotyped in 316 low-risk pregnant women from the general population in South Africa. Thirty-four (10.8%) of these women were subsequently diagnosed with late-onset pre-eclampsia. The remaining 282 samples served as controls. Genotype analysis revealed that the A/A genotype of 98A-C was detected in 67.7% of cases and 53% of controls. Heterozygosity (A/C) was detected in 14.7% of cases and 6.5% of controls. The control frequencies were in Hardy–Weinberg equilibrium, while the pre-eclampsia cases deviated significantly from equilibrium (Bruiners et al., 2007). The increased frequency of heterozygosity (A/C genotype) in the controls is highly suggestive of a protective effect against developing pre-eclampsia and warrants further investigation.

While pre-eclampsia is currently not considered a genetic disorder, the studies of Sammar et al. (2006), Bosman et al. (2008) and Gebhardt et al. (2009) indicate that there is a genetic predisposition to pre-eclampsia among patients with certain mutations in the LGALS13 gene or its splice variants.
Functional characterization of PP13

Localization of PP13 in the placenta

PP13 can be found at the apical membrane of the syncytiotrophoblast in placental sections obtained at delivery, while there is no staining of the villous cytotrophoblast (Than et al., 2004, 2008a; Huppertz et al., 2008) (Fig. 1C–F).

Kliman et al. (2012) further investigated labeling in placental and deciduous tissues of placentas between 7 and 15 weeks of GA. These authors found staining for PP13 on the surface of the villous syncytiotrophoblast as well as syncytiotrophoblast that covers trophoblastic cell columns. However, villous and extravillous cytotrophoblast did not show any staining for PP13. Staining intensity of the syncytiotrophoblast was most intense at 6–7 weeks and decreased at 12–15 weeks. In the decidua PP13 is mainly associated with early interaction with immune cells. Kliman et al. (2012) have put forward a hypothesis suggesting that PP13-induced apoptosis of immune cells far from spiral arteries and near uterine veins supports uninterrupted invasion of extravillous trophoblasts into spiral arteries. This hypothesis takes into account the proposal of Than et al. (2009) that galectins such as PP13 reduce the danger of maternal immune attack on fetal components by apoptosis of T-lymphocytes to confer immune tolerance.

Release of PP13

PP13 does not have a signaling sequence that allows it to be transported across the plasma membrane. However, it is secreted from the syncytiotrophoblast into the maternal circulation and can already be detected in maternal serum at week 5 GA (Huppertz et al., 2008). By cross-linking, peptide mapping and sequencing, Than et al. (2004) found that PP13 interacts with high affinity with annexin II and beta/gamma actin. This interaction is supposed to enable transport of PP13 across the plasma membrane via a calcium-mediated process (Burger et al., 2004). Balogh et al. (2011) found that the actin cytoskeleton, probably in connection with lipid rafts, controls trophoblastic ‘non-classical’ PP13 export.

A PP13-dependent calcium liberation from internal reservoirs into the cytoplasm of trophoblast cells has been detected (Burger et al., 2004). This process is further augmented by calcium permeability through the trophoblast outer membrane, as was demonstrated by Balogh et al. (2011). Using immortalized trophoblast cell lines transfected with PP13, these authors were able to show an increased PP13 release when the cells were exposed to a calcium ionophore. Calcium mobilization and its interaction with actin are most likely responsible for PP13 trafficking from inside the trophoblast to the plasma membrane and its liberation to the intervillous space.

PP13 and prostaglandins

PP13 has a mild lysophospholipase A activity (Than et al., 1999). Analysis of the intrinsic enzyme activity revealed that PP13 slowly converts the triglyceride backbone of plasma membrane phospholipids, leading to the release of free fatty acids within 18–72 h after exposure to purified PP13 (Burger et al., 2004; Than et al., 2004).

Burger et al. (2004) found that PP13 via its intrinsic mild phospholipase A activity leads to liberation of linoleic and arachidonic acids from trophoblasts followed by their conversion into prostacyclin and thromboxane, both acting as vasoactive prostaglandins. Accordingly, it was postulated that PP13 may regulate blood pressure of the uterine vasculature and from the placenta. Hence, the role of PP13 could be dual: (i) PP13 supports tolerance by apoptosis of decidua leukocytes, acting as an agent to reduce the immune response from invading trophoblasts (Kliman et al., 2012) and (ii) PP13 may prepare the utero-placental vessels to the needs of pregnancy, an action that may extend to other parts of the maternal body since PP13 is systemically present in maternal blood.

Longitudinal assessment of PP13 in vivo and in vitro

Normally, serum PP13 slowly increases during pregnancy and shows double-to-triple values close to delivery, after which it disappears from maternal blood within 2–5 weeks (Huppertz et al., 2008). In contrast, in the first trimester in women developing pre-eclampsia later in pregnancy, serum PP13 is significantly lower from normal (Fig. 2). This has been shown already at 5–7 weeks of gestation (Gonen et al., 2008; Huppertz et al., 2008). As pre-eclampsia progresses the level increases, most likely through the increased shedding of STBM, that convey a high concentration of PP13 into maternal blood. The sharper the increase of PP13 from first to third trimester, the more severe the anticipated pre-eclampsia symptoms (Gonen et al., 2008; Huppertz et al., 2008).

Explants obtained from placental villi have been extensively used for in vitro assays to study differentiation, transport, metabolism and endocrine functions (Miller et al., 2005). In such tissue cultures PP13 release reaches a steady state within 24 h, lasting for 48 h, and then slowly diminishes due to culture deterioration (Grimpel et al., 2011). PP13 release was higher from villous explants of term pre-eclampsia cases compared with term controls mimicking the higher maternal blood levels in pre-eclampsia at delivery compared with normal outcome. Interestingly, villous explants from first trimester placentas showed a 2-fold increased release of PP13 compared with term controls, similar to the values of term pre-eclampsia cases. The findings indicate a much higher PP13 release rate per trophoblast surface unit in the first trimester and may be associated with a greater need to support tolerance of the mother in the first compared with the third trimester (Grimpel et al., 2011).

Pre-eclampsia prophylaxis

Indications from in vitro models

As described above, the villous explant model predicts that in the first trimester high levels of PP13 are important for progression of pregnancy without development of pre-eclampsia. Hence, elevation of PP13 in the first trimester in cases with very low PP13 (those at high risk to develop pre-eclampsia) could act as a putative prophylaxis for pre-eclampsia, while its reduction in the third trimester could serve this function as well (Grimpel et al., 2011). A similar scenario for the levels in the third trimester has been described for sFlt-1 (Thadhani et al., 2011).

Using the trophoblast-derived cell line BeWo treated with forskolin to induce fusion, it has been shown that PP13 and beta-hCG proteins and their corresponding mRNAs, were significantly increased under conditions forcing syncytial fusion (Orendi et al., 2010, 2011). This model was used as a surrogate for first trimester villous trophoblast. Testing putative therapeutic drugs such as vitamins C and E, low-molecular weight heparin (LMWH) and aspirin, dose-dependent changes of PP13 and/or...
beta-hCG were detected after stimulation. Hence, specific drugs may well be suitable as prophylaxis for women with low maternal blood PP13 in the first trimester of pregnancy (Orendi et al., 2011).

**Putative prophylactic treatments in vivo and in vitro**

**MgSO4:** The MagPie multicenter study (Altman et al., 2002) has shown that venous infusion of magnesium sulphate 24 h prior to delivery can reduce the frequency of eclampsia but not of pre-eclampsia. *In vitro*, magnesium sulphate reduced PP13 release from placental explants obtained after delivery from normal pregnancy. However, magnesium sulphate increased PP13 release when explants were obtained from pre-eclampsia cases. Magnesium sulphate reduced PP13 release to a higher extent in first trimester explants. Magnesium chloride gave similar results, indicating that it is the magnesium not the sulphate that is responsible for the adverse effects on PP13 release from trophoblast *in vitro* (Grimpel et al., 2011).

**CaCl2:** Calcium supplementation has been shown to reduce the frequency of pre-eclampsia among women from South America and other countries, where nutrition habits involve a relatively low consumption of dairy products (Hofmeyr et al., 2006; Seely, 2007). The WHO has recommended 1–2 g/day calcium supplementation as a prophylaxis for pre-eclampsia (WHO, 2011). Exposing term villous explants to increasing calcium concentrations showed a tendency to decreased PP13 release in explants from controls and more prominent decreases from pre-eclampsia cases (Grimpel et al., 2011). In the first trimester moderate calcium concentrations increased PP13 release but at higher concentrations they attenuated PP13 release significantly. The effect of calcium was blocked when EDTA was added (Grimpel et al., 2011). Accordingly, calcium may have its value as a prophylaxis in both first and third trimester under a very strict blood level control to maintain the required value.

**LMWH:** low-molecular weight heparin (LMWH) is a common drug used as an anti-coagulant. Correlations between genetic mutations in various factors involved in blood coagulation were found in patients at high risk to develop pre-eclampsia (Kupferminc et al., 2001; Dodd et al., 2010). LMWH was recently implicated in apoptosis and immune suppression (Rimon, 2012). Adding LMWH to villous explants had marginal effects with a tendency to reduce PP13 release in unaffected term controls. In both pre-eclampsia and first trimester explants LMWH decreased PP13 release. The results indicated that LMWH is not suitable to serve as a prophylaxis in first trimester but may be beneficial near or at the time of symptoms (Grimpel et al., 2011). Similarly, in BeWo cells LMWH did not show any effect on PP13 and beta-hCG levels (Orendi et al., 2010).

Aspirin: taking aspirin, especially if started before 16 weeks, is associated with a 50% decreased frequency of pre-eclampsia (Vainio et al., 2002; Bujold et al., 2009). The concentration range usually used is 75–130 mg/day, which was estimated to be better compared with lower doses of 65–100 mg/day (Bujold et al., 2009). Other studies have indicated that increasing the dose to 100–300 mg/day may be associated with an increased frequency of placental abruption (Sibai et al., 1993).

Aspirin slightly decreased PP13 release from villous explants of unaffected term placentas. A bell-shaped curve was seen in explants derived from pre-eclampsia cases and first trimester placentas, with increased PP13 release at moderate aspirin concentrations, which returned to normal at high aspirin concentrations. These results show a very strong dose dependence of PP13 release when using aspirin, especially in pre-eclampsia and first trimester (Grimpel et al., 2011). Hence, from the villous explant model it appears as if aspirin is the most favorable prophylaxis in the first trimester but may not be beneficial toward delivery.

In BeWo cells aspirin reduced PP13 to 60% of the respective control, while there was no effect on beta-hCG (Orendi et al., 2010). Also, aspirin increased the fusion rate, indicating its favorable effect on villous trophoblast maturation. These results provide evidence that aspirin may support the development of placental villi early in pregnancy by stimulating fusion but is not involved in directly increasing PP13.

**Vitamins C and E:** these two vitamins are potent antioxidants. However, a number of randomized placebo–control clinical studies showed that their supplementation either had no effect or even increased severity of several aspects of pre-eclampsia (Poston et al., 2006; Rumbold et al., 2006).

**Vitamin C:** in BeWo cells PP13 mRNA as well as beta-hCG levels were significantly increased after supplementation with physiological concentrations of vitamin C (Orendi et al., 2010). According to *in vivo* and *in vitro* data, vitamin C may have a narrower window of positive action than initially anticipated. It may be beneficial in the first trimester by elevating PP13 and beta-hCG to support placentation. However, if used later in pregnancy, it may be ineffective or even become dangerous.

**Vitamin E:** In BeWo cells supplementation of the vitamin E derivative Trolox at concentrations above the physiological level stimulated fusion; however, at the physiological range vitamin E had no effect on fusion or PP13 and beta-hCG mRNA levels (Orendi et al., 2010).
Promoting the risk for pre-eclampsia with PP13: a meta-analysis

Immunohistochemistry

PP13-specific mouse monoclonal antibodies (MAb) were generated and a pair of immunoglobulin G1 antibodies was selected as products for immune diagnostics (MAb 27-2-3 and MAb 215-28-3) due to their very high affinity to native and recombinant PP13 (Burger et al., 2004). Both antibodies identify wild-type PP13 (Burger et al., 2004; Sammar et al., 2011) in western blots, but do not recognize the mutated DelT221 or Dex 2 variants that are recognized by polyclonal antibodies against PP13 (Sammar et al., 2006).

The enzyme-linked immunosorbent assay (ELISA) format for the detection of PP13 in body fluids uses MAB 27-2-3 as the capture antibody and MAB 215-28-3 as the detection antibody coupled to biotin. Reaction specificity of the ELISA is evident against non-pregnant and male serum. The linear range of the ELISA is between 12.5 and 400 pg/ml PP13, the lower detection level is 3–8 pg/ml and the kit-to-kit, operator-to-operator and batch-to-batch variations are all in the range of 3–12% (Burger et al., 2004) or higher (Romero et al., 2008a, b).

The Delfia format was developed later with MAB 215-28-3 as the capture antibody and MAB 27-2-3 as the detection antibody. The linear range of the Delfia is between 0 and 2000 pg/ml PP13 and above, the lower detection level is 1–3 pg/ml and the kit-to-kit, operator-to-operator and batch-to-batch variations are all below 6% (Cowan et al., 2011).

Methods and included studies for the PP13 meta-analysis

There are 68 studies on PP13 and 19 out of 68, published between January 2006 and September 2012, used the two above immunohistoassays to evaluate maternal blood PP13 as a marker of pre-eclampsia (Table I). Studies on in vitro systems, immune labeling, reviews and overviews were omitted. The evaluation was performed using Forest plot for meta-analysis (Lewis and Clarke, 2001). Longitudinal studies of Huppertz et al. (2008) and Gonen et al. (2008) have shown that around midgestation women who will develop pre-eclampsia show a steep increase in serum PP13, indicating entry into the active stage of the disorder. However, the small number of studies in the second and third trimester (e.g. Spencer et al., 2007a) precludes further meta-analysis.

The DR at 10% FPR of the 19 studies was extracted from the published data’s ROC curves or by communicating with the authors. The 95% confidence interval (95% CI) of the DR was extracted from ROC curves or calculated with a web-calculator (http://www.causascientia.org/math_stat/ProportionCI.html). The analysis pooled clinical results from all singleton prospective nested-case/control studies or full prospective studies (including longitudinal studies) that enrolled low and high risk all-comers. The analysis initially pooled all pre-eclampsia cases, and then subdivided to term, intermediate and early pre-eclampsia. Cases with pure gestational hypertension were excluded.

The studies had an international scope involving the UK (Nicolaidis et al., 2006; Spencer et al., 2007b; Khalil et al., 2009; Akolekar et al., 2009, 2011), the USA (Chafetz et al., 2007; Romero et al., 2008b; Odibo et al., 2011; Than et al., 2011; Myatt et al., 2012), Canada (Audibert et al., 2010), Germany (Huppertz et al., 2008), Italy (Di Lorenzo et al., 2012), Middle East (Gonen et al., 2008; El Sherbiny et al., 2012; Moslemi-Zadeh et al., 2012; Svirski et al., 2013), Australia (Schneuer et al., 2012), China (Odibo et al., 2011), and the Netherlands (Wortelboer et al., 2010). The study of Saharanand et al. (2011) was used as an indicator when PP13 is first detected in maternal blood. Kuc et al. (2011), Khalil et al. (2010), Akolekar et al. (2011) and Cuckle (2011) were used as multi-marker analysis studies and Cowans et al. (2011) used a different ELISA based on an assay developed in China was also included in the analysis. Blood samples were collected at gestational weeks 6–14, with 80% of the samples from 10 to 12 weeks.

Adjustment to MoM: In all studies PP13 blood levels measured by the PP13 assays were converted to gestational week specific multiples of the medians (MoMs), further adjusted to BMI (usually) or maternal parity. In one study the values were further adjusted to conception by IVF. Results with PP13: in all first trimester studies maternal blood PP13 level was lower in women who subsequently developed pre-eclampsia, although the range of differences was huge. The prediction by PP13 has a very broad range (17–91%) (Table I). The mean DR for 10% FPR for all pre-eclampsia cases was 47% (95% CI: 43–65) (Fig. 3A). The difference may be attributed to the proportion of severe versus mild cases in each study, since in one study the mild cases were shown not to have significantly different PP13 levels compared with controls (Romero et al., 2008a, b). The sensitivity for intermediate and early pre-eclampsia combined was much higher, 66%...
### Table I All preeclampsia cases: ELISA and Delfia.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Study design</th>
<th>Population</th>
<th>PE</th>
<th>All patients</th>
<th>DR (% 95% CI)</th>
<th>ELISA (E)/Delfia (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicolaides et al.</td>
<td>2006</td>
<td>NCC</td>
<td>All comers</td>
<td>24</td>
<td>447</td>
<td>44 (33–53)</td>
<td>E</td>
</tr>
<tr>
<td>Chafetz et al.</td>
<td>2007</td>
<td>NCC</td>
<td>All comers</td>
<td>47</td>
<td>425</td>
<td>79 (64–89)</td>
<td>E</td>
</tr>
<tr>
<td>Spencer et al.</td>
<td>2007a,b</td>
<td>NC</td>
<td>All comers</td>
<td>88</td>
<td>536</td>
<td>55 (45–64)</td>
<td>E</td>
</tr>
<tr>
<td>Gonen et al.</td>
<td>2008</td>
<td>Prospective</td>
<td>Low risk</td>
<td>20</td>
<td>1239</td>
<td>69 (57–79)</td>
<td>E</td>
</tr>
<tr>
<td>Huppertz et al.</td>
<td>2008</td>
<td>Prospective</td>
<td>Low risk</td>
<td>4</td>
<td>63</td>
<td>91 (80–100)</td>
<td>E</td>
</tr>
<tr>
<td>Romero et al.</td>
<td>2008a,b</td>
<td>NCC</td>
<td>Low risk</td>
<td>50</td>
<td>300</td>
<td>65 (58–73)</td>
<td>E</td>
</tr>
<tr>
<td>Khalil et al.</td>
<td>2009</td>
<td>NCC</td>
<td>High Risk</td>
<td>42</td>
<td>252</td>
<td>62 (44–78)</td>
<td>E</td>
</tr>
<tr>
<td>Akolekar et al.</td>
<td>2009</td>
<td>Prospective</td>
<td>All comers</td>
<td>48</td>
<td>624</td>
<td>38 (24–53)</td>
<td>D</td>
</tr>
<tr>
<td>Wortelboer et al.</td>
<td>2009</td>
<td>NCC</td>
<td>All comers</td>
<td>88</td>
<td>568</td>
<td>36 (27–57)</td>
<td>D</td>
</tr>
<tr>
<td>Odbo et al.</td>
<td>2011</td>
<td>Prospective</td>
<td>All comers</td>
<td>42</td>
<td>452</td>
<td>45 (33–59)</td>
<td>D</td>
</tr>
<tr>
<td>Audibert et al.</td>
<td>2010</td>
<td>Prospective</td>
<td>All comers</td>
<td>40</td>
<td>893</td>
<td>19 (7–27)</td>
<td>D</td>
</tr>
<tr>
<td>Akolekar et al.</td>
<td>2011</td>
<td>Prospective</td>
<td>All comers</td>
<td>221</td>
<td>1534</td>
<td>42 (29–61)</td>
<td>D</td>
</tr>
<tr>
<td>Than et al.</td>
<td>2011</td>
<td>Prospective</td>
<td>Low risk</td>
<td>20</td>
<td>1078</td>
<td>70 (65–77)</td>
<td>E</td>
</tr>
<tr>
<td>El Sherbiny et al.</td>
<td>2012</td>
<td>NCC</td>
<td>All comers</td>
<td>50</td>
<td>50</td>
<td>86 (78–93)</td>
<td>E</td>
</tr>
<tr>
<td>Di Lorenzo et al.</td>
<td>2012</td>
<td>Prospective</td>
<td>All comers</td>
<td>25</td>
<td>2018</td>
<td>30 (15–45)</td>
<td>D</td>
</tr>
<tr>
<td>Moslemi-Zadeh et al.</td>
<td>2012</td>
<td>NCC</td>
<td>Low risk</td>
<td>100</td>
<td>1500</td>
<td>77 (72–82)</td>
<td>E</td>
</tr>
<tr>
<td>Myatt et al.</td>
<td>2012</td>
<td>NCC</td>
<td>Low risk</td>
<td>174</td>
<td>509</td>
<td>17 (11–25)</td>
<td>D</td>
</tr>
<tr>
<td>Svirski et al.</td>
<td>2013</td>
<td>Prospective</td>
<td>High risk</td>
<td>43</td>
<td>676</td>
<td>69 (61–78)</td>
<td>E</td>
</tr>
<tr>
<td>Schneuer et al.</td>
<td>2012</td>
<td>Prospective</td>
<td>All comers</td>
<td>71</td>
<td>2989</td>
<td>34 (23–46)</td>
<td>D</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>Meta analysis</td>
<td></td>
<td>1197</td>
<td>16 153</td>
<td>47 (43–65)</td>
<td></td>
</tr>
</tbody>
</table>

The numbers on the left side correspond to the graph numbers in Fig. 3A. The table lists all studies used to perform the Forest plot for the meta-analysis of all cases of pre-eclampsia (PE). The DR (DR) for a 10% FPR is shown along with the 95% confidence interval (CI). E and D refer to the test assays (E, ELISA; D, Delfia). Under study design NCC is a nested case–control study. The forest plot also contains information on the population analyzed in the studies (all comers or low risk or high risk) and whether the study was a nested case–control study. The Forest analysis is based on providing respective weight for studies based on the number of pre-eclampsia cases as well as the total number of women in the study.

(95% CI: 48–78) (Table II) (Fig. 3B). Sensitivity for early cases alone was 83% (95% CI: 25–100) (not shown).

Assays: at 10% FPR, the DR with the ELISA assay was higher compared with the Delfia assay for all cases as well as intermediate and early cases (Fig. 3). A comparative analysis of the same samples with the two assays indicated a small decrease in sensitivity for Delfia compared with ELISA (Cowans et al., 2011). However, the ELISA/Delfia comparison of Cowans et al. (2011) cannot explain data of two studies where the Delfia DR was very poor (Di Lorenzo et al., 2012; Myatt et al., 2012). Such low DRs in the above two studies may be derived from differences in the definition of preeclampsia, or, more likely, differences in blood drawing, processing, and especially sample storage.

PP13 is challenging the immune-diagnostic procedures since its blood level is in the picogram range. This picodose of PP13 could be influenced by its high affinity to sugar residues of various blood proteins and particularly the sugar residues of red blood cells (Than et al., 2011). PP13 can oligomerize to form dimers and oligomers and subsequently become non-available to the immunoassay. Hence, DTT is used as a reducing agent in the sample dilution buffer of the ELISA but not the Delfia assay to keep PP13 in its monomer configuration. This difference between ELISA and Delfia may influence antibody affinity and the level of free PP13 determined in blood while decreasing non-specific binding.

In conclusion: the meta-analysis indicates that first trimester blood PP13 of pregnant women is lower in the first trimester among women who will subsequently develop pre-eclampsia. Further development and stabilization of both the ELISA and Delfia assays are required to achieve more robust data. Additionally, scientists need to set high value on the standardization of blood tubes, centrifugation and storage conditions of serum samples. A 30% variation in serum PP13 level can be shown for the gel vacutainer standardized for the separation of serum from blood clots compared with non-gel containing blood tubes (H. Meiri, unpublished data).

### Multiple markers

Prediction of pre-eclampsia is improved when combining multiple markers. In an algorithm combining PP13 into a logistic regression with patients’ pregnancy history, demography and current pregnancy disease, PP13 prediction provided added accuracy over background risk (Akolekar et al., 2009). Combining PP13 with PIGF (Wortelboer et al., 2010) or with additional markers (Cuckle, 2011) increased the DR. The apparent reduction of PP13 in the first trimester among IVF patients (Svirski et al., 2013) is a recent finding that warrants further testing and evaluation. Nicolaides et al. (2006) and Spencer et al. (2007a,b) showed increased prediction accuracy of PP13 with the Doppler pulsatility index of uterine arteries. Combining PP13 with
Doppler ultrasound and maternal artery stiffness increased the DR to 93% for early and 86% for all pre-eclampsia cases at 10% FPR. Recently, Gonen et al. (2013) have found improved prediction when combining PP13 with patient demography and history and with mean arterial blood pressure at the time of testing, reaching 92% DR at 12% FPR. Accordingly, combined with the background risk derived of pregnancy, medical history and additional markers, pre-eclampsia prediction using PP13 could satisfy the WHO requirements for an effective marker.

Multi-parametric approaches combining variables of different methodologies have a higher predictive value for pre-eclampsia than individual markers. Combining maternal history, demographics, mean arterial pressure, Doppler ultrasound, cardiovascular measures and serum markers, Poon et al. (2013) and Parra-Cordero et al. (2012) found very high predictive values for early, intermediate and even for term pre-eclampsia and paved the way to adopt this approach for effective clinical screening.

From individualized risk to drug development

The concept

The field of novel biological drug development has been significantly modified following the introduction of molecular biomarkers not only for diagnosis but as main agents in directing prevention and treatment. The approach revolutionized medical management and started the successful implementation of personalized medicine, which is evident in treatments such as managing cancer progression by blocking HER 1&2 receptors.

The new concept for pre-eclampsia relies on pilot experimental results that indicate that patients at risk for developing pre-eclampsia who have low first trimester PP13 may benefit from replenishing PP13 (Gizurarson et al., 2013).

Hypothesis: Keeping PP13 levels within a ‘therapeutic window’ is essential to prevent pre-eclampsia

It has been suggested that PP13 is involved in a multi-step process that supports trophoblast invasion as well as the generation of a systemic endothelial effect within the mother (Visegrady et al., 2001; Than et al., 2004; Kliman et al., 2012). Women with low PP13 levels in the first trimester may lack the necessary amount of this factor to enable immune tolerance and to prepare the maternal vasculature for the increased blood flow needed to supply the fetus during the second half of pregnancy. If impaired placental development (low PP13) does not lead to endothelial adaptation or pre-conditioning in the first trimester, an increased release of placental factors will negatively affect the maternal endothelium in the second half of pregnancy. This may in turn lead to the clinical symptoms of pre-eclampsia. Accordingly, replenishing PP13 early in pregnancy may provide a novel therapeutic basis to reverse or limit the impact of impaired placental-associated with the development of pre-eclampsia. The hypothesis of replenishing PP13 for keeping its levels within a ‘therapeutic window’ to prevent pre-eclampsia requires further safety studies considering that not all patients with low PP13 will develop pre-eclampsia. As in many other examples, in some women compensatory mechanisms develop to maintain a normal

**Pilot study**

The following data are taken from a recent publication showing data of a pilot study on the effects of PP13 on gravid rats (Gizurarson et al., 2013).

**Single dosage PP13:** a single dose of human PP13 was administered to gravid rats, which lead to an immediate reduction in arterial blood pressure accompanied by an increase in the heart rate. Control animals exposed to heat-inactivated PP13 showed no changes in blood pressure or heart rate.

**Vasodilation:** administration of PP13 on isolated arteries resulted in arterial dilation. This PP13 induced vasodilation could not be further increased during this period by adding vasodilators such as papaverine, indicating that PP13 may either bind to the same receptors or stabilize the artery in a maximally dilated stage.

**Continuous delivery of PP13 over 5 days:** to follow up on the single bolus study, gravid rats who received PP13 continuously from days 15 to 20 of pregnancy (term = day 22) were compared with saline-infused controls. Blood pressure during this time was significantly lower compared with control animals. At the same time, heart rate increased significantly in the PP13 group indicating that a general vasodilation had occurred, reducing peripheral resistance.

---

**Table II Early and intermediate preeclampsia: ELISA and Delfia.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Study design</th>
<th>Population</th>
<th>PE</th>
<th>All patients</th>
<th>DR (95% CI)</th>
<th>ELISA (E)/Delfia (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 Nicolaides et al.</td>
<td>2006</td>
<td>NCC</td>
<td>All comers</td>
<td>10</td>
<td>433</td>
<td>80 (44–98)</td>
<td>E</td>
</tr>
<tr>
<td>17 Chafetz et al.</td>
<td>2007</td>
<td>NCC</td>
<td>All comers</td>
<td>3</td>
<td>425</td>
<td>92 (84–100)</td>
<td>E</td>
</tr>
<tr>
<td>16 Spencer et al.</td>
<td>2007a, b</td>
<td>NCC</td>
<td>All comers</td>
<td>44</td>
<td>536</td>
<td>71 (63–89)</td>
<td>E</td>
</tr>
<tr>
<td>15 Gonen et al.</td>
<td>2008</td>
<td>Prospective</td>
<td>Low risk</td>
<td>5</td>
<td>1239</td>
<td>80 (71–89)</td>
<td>E</td>
</tr>
<tr>
<td>14 Romero et al.</td>
<td>2008a, b</td>
<td>NCC</td>
<td>Low risk</td>
<td>13</td>
<td>300</td>
<td>77 (64–89)</td>
<td>E</td>
</tr>
<tr>
<td>12 Akolekar et al.</td>
<td>2009</td>
<td>Prospective</td>
<td>All comers</td>
<td>48</td>
<td>624</td>
<td>52 (42–75)</td>
<td>D</td>
</tr>
<tr>
<td>11 Wortelboer et al.</td>
<td>2009</td>
<td>NCC</td>
<td>All comers</td>
<td>88</td>
<td>568</td>
<td>36 (27–57)</td>
<td>D</td>
</tr>
<tr>
<td>10 Odbo et al.</td>
<td>2011</td>
<td>Prospective</td>
<td>All comers</td>
<td>12</td>
<td>452</td>
<td>68 (53–79)</td>
<td>D</td>
</tr>
<tr>
<td>9 Audibert et al.</td>
<td>2010</td>
<td>Prospective</td>
<td>All comers</td>
<td>9</td>
<td>893</td>
<td>29 (17–37)</td>
<td>D</td>
</tr>
<tr>
<td>8 Akolekar et al.</td>
<td>2011</td>
<td>Prospective</td>
<td>All comers</td>
<td>118</td>
<td>1431</td>
<td>46 (32–85)</td>
<td>D</td>
</tr>
<tr>
<td>7 Than et al.</td>
<td>2011</td>
<td>Prospective</td>
<td>Low risk</td>
<td>5</td>
<td>1078</td>
<td>85 (78–93)</td>
<td>E</td>
</tr>
<tr>
<td>6 Di Lorenzo et al.</td>
<td>2012</td>
<td>Prospective</td>
<td>All comers</td>
<td>12</td>
<td>2018</td>
<td>15 (5–45)</td>
<td>D</td>
</tr>
<tr>
<td>5 Moslemi-Zadeh et al.</td>
<td>2012</td>
<td>NCC</td>
<td>Low risk</td>
<td>11</td>
<td>1500</td>
<td>91 (85–96)</td>
<td>E</td>
</tr>
<tr>
<td>4 Myatt et al.</td>
<td>2012</td>
<td>NCC</td>
<td>Low risk</td>
<td>99</td>
<td>509</td>
<td>22 (11–45)</td>
<td>D</td>
</tr>
<tr>
<td>3 Svirski et al.</td>
<td>2013</td>
<td>Prospective</td>
<td>High risk</td>
<td>14</td>
<td>676</td>
<td>79 (71–88)</td>
<td>E</td>
</tr>
<tr>
<td>2 Schneuer et al.</td>
<td>2012</td>
<td>Prospective</td>
<td>All comers</td>
<td>5</td>
<td>2989</td>
<td>60 (15–95)</td>
<td>D</td>
</tr>
<tr>
<td>1 Total</td>
<td></td>
<td></td>
<td></td>
<td>532</td>
<td>15 923</td>
<td>66 (48–78)</td>
<td></td>
</tr>
</tbody>
</table>

The numbers on the left side correspond to the graph numbers in Fig. 3B. The table lists all studies used to perform the Forest plot for the meta-analysis of early and intermediate cases of pre-eclampsia. The DR (DR) for a 10% FPR is shown along with the 95% confidence interval (CI). E and D refer to the test assays (E, ELISA; D, Delfia). The table also contains information on the population analyzed in the studies (all comers or low risk or high risk) and whether the study was a nested case–control or a prospective study. The Forest analysis is based on providing respective weight for studies based on the number of pre-eclampsia cases as well as the total number of women in the study.

---

**Could PP13 aid in preventing pre-eclampsia?**

Could PP13 replenishment be used to treat/prevent pre-eclampsia? PP13 is limited to anthropoids/simians, a group of primate species that differ from other primates by having relatively large brains and a long gestation (Than et al., 2009). If the beneficial effects of PP13 are further verified by *in vitro* and animal studies, therapeutic treatment with PP13 may enter clinical studies, which would allow one to test the potential benefits of replenishment. Acting in the picogram range, this may require combining PP13 with nanomaterials and delivery systems to avoid adherence, aggregation, and loss in the bloodstream. Optimally, women would first be screened for their risk of developing pre-eclampsia as defined by the shortage of PP13 either alone or in combination with other markers that were discussed above. Finally, it needs to be verified that there is no harm to the fetus. This can be achieved by better understanding how the placenta handles PP13, and further identifying the spectrum of action of PP13 at the cellular and tissue level. Will it be possible to give PP13 to pregnant women, more specifically during the embryonic period of organogenesis which is the most critical time of pregnancy? Although there are undeniable challenges ahead, a closer examination of the therapeutic value of PP13 is warranted to develop the prevention of pre-eclampsia, the most widespread and morbid gestational disorder, and one that is: in spite of many years of intensive study; still awaiting a new approach to identify an effective medical treatment.
Acknowledgements

The authors thank Prof. Ron Gonen, Bnai Zion Medical Center, and Rappoport Faculty of Medicine, Technion, Haifa, Israel for his continuous collaboration on a number of clinical studies on PP13. The authors also thank Dr Kristina Orendi for providing information related to in vitro systems and immunostaining at the Medical University of Graz. We thank the former employees of Diagnostic Technologies, Dr Ora Burger, Dr Elah Pick-Golan, Dr Ilana Chefetz, Dr Vered Kivity, Yael-Inna Grimpel, Galina Fihman, Boris Rappaport and Haia Tal for providing information related to PP13 and clinical studies. The authors thank Drs Reneate Hillerman and George Rebello, Cape Town, South Africa for the collaborative work on PP13 mutations. We also thank Hananja ehf for funding part of the work. The authors thank Drs Renate Hillerman and George Rebello, Cape Town, South Africa for the collaborative work on PP13 mutations. We also thank Hananja ehf for funding part of the work. The authors thank Dr Ora Burger, Dr Elah Pick-Golan, Dr Ilana Chefetz, Dr Vered Kivity, Yael-Inna Grimpel, Galina Fihman, Boris Rappaport and Haia Tal for providing information related to PP13 and clinical studies. The authors thank Drs Renate Hillerman and George Rebello, Cape Town, South Africa for the collaborative work on PP13 mutations.

Authors’ roles

All parties were involved in preparing this manuscript and overall data analysis. M.S., H.M. and B.H. were main contributors to the description of PP13. S.M. performed the recombinant PP13 work with the wild type and mutations of PP13 and B.H. contributed to original staining images. H.M. and B.H. performed the meta-analysis and S.G. and G.O. carried out the pilot studies in rat in collaboration with S.M., H.M. and B.H.

Funding

Many of the studies published here were sponsored by the EU FP6-project “Pregenesys” (FP6, #37244).

Conflict of interest

H.M. and M.S. were employees of Diagnostic Technologies limited (DTL) who made PP13 kits until the company stopped its operation in 2011. H.M. is a paid consultant to Hy-Laboratories that acquired the rights to the PP13 technology. H.M. and S.G. own patent rights to the use of PP13 as a drug. Otherwise, all authors declared no conflict of interest while analyzing the results and writing this manuscript.

References


Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension 2008; 51:970 – 975.


PP13: from pre-eclampsia prediction to prevention


