JOURNAL OF CIRCULATING BIOMARKERS

Journal of Circulating Biomarkers
Volume 7: 1–16

DOI: 10.1177/1849454418786159
journals.sagepub.com/home/cbx
SAGE

Placental protein 13: An important biological protein in preeclampsia

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Abstract

Placental protein 13 (PP13), a glycan binding protein predominantly expressed in syncytiotrophoblast, dimeric in nature, lacks N-terminal signal peptide, bypasses the endoplasmic reticulum, and secretes into maternal circulation as exosomes or microvesicles. PP13 has jelly roll fold conformation with conserved carbohydrate recognition domain which specifically binds to β-galactosides of the glycan receptors during placentation. PP13 binds to glycosylated receptors on human erythrocytes and brings about hemagglutination by the property of lectin activity; other functions are immunoregulation and vasodilation during placentation and vascularization. The gene LGALS13 located on 19q13.2 comprising four exons expresses a 32-kDa protein with 139 amino acid residues, PP13. Impaired expression due to mutation in the gene leads to a nonfunctional truncated PP13. The low serum levels predict high risk for the onset of preeclampsia or obstetric complications. Hence, PP13 turned to be an early marker for risk assessment of preeclampsia. The recombinant PP13 and monoclonal antibodies availability help for replenishing PP13 in conditions with low serum levels and for detection and prevention of preeclampsia, respectively.

Keywords

Placental protein 13, preeclampsia, eclampsia, jelly roll fold, syncytiotrophoblast

Date received: 30 December 2017; accepted: 28 May 2018

Introduction

Preeclampsia is a pregnancy-specific disorder characterized by hypertension and proteinuria after 20 weeks of gestation.[1] Although the exact etiology of the disorder is still not known, the impairment in the early placentation is associated with onset of preeclampsia that complicates up to 2–8% of all the pregnancies.[2] Prediction, understanding of underpinning mechanism and prevention of preeclampsia, is still not clear. Hence, preeclampsia and eclampsia are the leading causes of maternal, perinatal morbidity, and mortality.[3,4] Placental protein 13 (PP13) is a carbohydrate binding protein synthesized in the syncytiotrophoblast, which is involved in early placentation process.[5,6] It is a member of galectin family with a conserved carbohydrate recognition domain (CRD).[7–9] The specificity of this site for β-galactosides-containing glycoconjugates[10–12] is established and plays a significant role in biological events such as implantation and embryogenesis.[13,14] The biological specificity of the PP13 present in the apical membrane of the syncytiotrophoblast to the glycans of the membrane and extracellular matrix proteins such as annexin II is a primary requisite for the placental implantation to the endometrium.[15]

PP13 also binds to β- and γ-actin within trophoblasts, which facilitates the migration of trophoblasts toward the placental bed and also increases the release of prostacyclins for vascular remodeling of maternal spiral arteries in early placentation.[16] From the immunological point of view, for an effective placentation, PP13 induces the apoptosis of

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maternal T cells to progress the deepening of implantation.[17] The expression of PP13 is also important for differentiation and syncytialization of the villous trophoblast which is vital for the release of placental hormones and immune proteins for embryo development and immune tolerance.[14,18] During pregnancy, various factors are the reason for the release of PP13 into the maternal circulation. The available studies indicated the low levels of PP13 in serum/plasma generally in first trimester of pregnancy. However, the levels gradually increase as gestation progresses.[19–22] The exact reason for the decreased PP13 levels in first trimester and increased levels in the third trimester is not known which is in line with the etiology of the preeclampsia. Preeclampsia is associated with impaired placentation,[23,24] evidence of low serum levels of PP13, and other heterogeneous causes, hence became a major cause for morbidity and mortality of fetus and mother.[25,26] Therefore, an attempt was made to summarize the information on PP13 in this article.

Historical perspective

Hans Bohn (1928–2014) gets the credit for successful isolation of factor XIII (fibrin stabilizing factor) of blood coagulation system from human placenta. After discovery, he developed a quantification method to demonstrate factor XIII deficiency that affects fibrin cross-linkage and fibrin stabilizing property and is associated with wound healing after injury or surgery.[27] During isolation and purification procedure of factor XIII, he noticed that a side fraction yielded human placental lactogen. Throughout this period, there was a rise in the research interest related to physiological and pathological aspects of human placenta.[28] This trend motivated him to explore the content of human placenta from a biochemical perspective. The galectin generally is numbered sequentially and hence the names for galectin 1–12 were assigned.[29]

The new member of the galectin family PP13 has been designated as galectin 13.[28] PP13 is one among the 56 proteins isolated from the placenta by Hans Bohn. Later on, in 1983, a research contribution by his coworkers led to the purification and characterization of placental tissue proteins 13 and 17.[30] He characterized many PPs for their physicochemical characteristics and also developed immunoassay to quantify these proteins of diagnostic significance. Several PPs such as PP4 (annexin-V), PP5 (tissue factor pathway inhibitor-2), PP10 (plasminogen activator inhibitor-2), and PP13 (galectin 13) were studied for their amino acid sequence and biological functions with respect to biological regulators of pregnancy process.[31–33]

Thereafter, he continued his research in collaboration with other scientists to investigate sequencing, structural, and molecular biological characterization of several PPs including PP13. This research contribution significantly improved the understanding of biological role and diagnostic importance in pregnancy complication, malignancies, and other placental and pregnancy-related protein research. Subsequently, PP research continued to add more information for further discoveries and improvements in clinical diagnostics and patient care.

Biosynthesis, secretion, and physical and chemical characteristics of PP13

Biosynthesis of PP13

Biosynthesis of PP13 occurs on free ribosomes in the cytoplasm of syncytiotrophoblast and is present in soluble form in the brush border apical plasma membrane as evident by staining techniques.[34,35] The placental gene *LGALS13* located on the long arm of chromosome 19 at loci q13.2 encodes PP13 protein exclusively.[36] *LGALS13* gene and its transcribing unit codes for PP13 at 5′ end that has promoter region followed by comprising four exons designated as E1–E4 (Figure 1). The full length of cDNA encoding human placental PP13 from expression library (Gen bank; acc. no: AF117383.1) predicted the molecular mass and the amino acid composition of the cloned protein which corresponds to 578 bp insert with a 417-bp open reading frame encoding a 139-amino acid protein.[37,38]

Secretion of PP13

Nascent PP13 lacks N-terminal signal sequence; hence, it bypasses the translocation route to endoplasmic reticulum and golgi bodies. Instead, PP13 utilizes the "non-classical" secretory pathway or unconventional routes to reach the maternal circulation either through vesicular shedding or direct translocational system. Studies showed that in the syncytiotrophoblast, PP13 is highly co-localized with cytoskeletal protein actin, annexin II, placental alkaline phosphatase, a glycosylphosphatidylinositol-anchored lipid raft resident protein, and CD71, a non-raft plasma membrane protein.[28] The actin cytoskeleton polymerization and associated motor proteins are the driving force for variety of cellular processes for transportation of galectins.[39,40] The various secretory routes utilized by galectins in the syncytiotrophoblast are either direct translocational or extra cellular vesicular transport in the form of microvesicles (40–100 nm) and exosomes (0.1–1 μm).[41–44] A lot has been already published on the release of PP13 by shedding syncytiotrophoblast microparticles.[28,35] A recent study has indicated that the amount of PP13 released via the exosomes and microvesicles is actually decreased in preeclampsia. The process regulating the release of these organelles has not been extensively investigated and is not well known. Therefore, future studies must evaluate PP13 biomarker potential in association with syncytiotrophoblast extracellular vesicles and exosomes.[45]

Even though PP13 primarily originated from placenta, it has been demonstrated that its expression was also noticed in human healthy liver, spleen, kidney, and bladder tissues

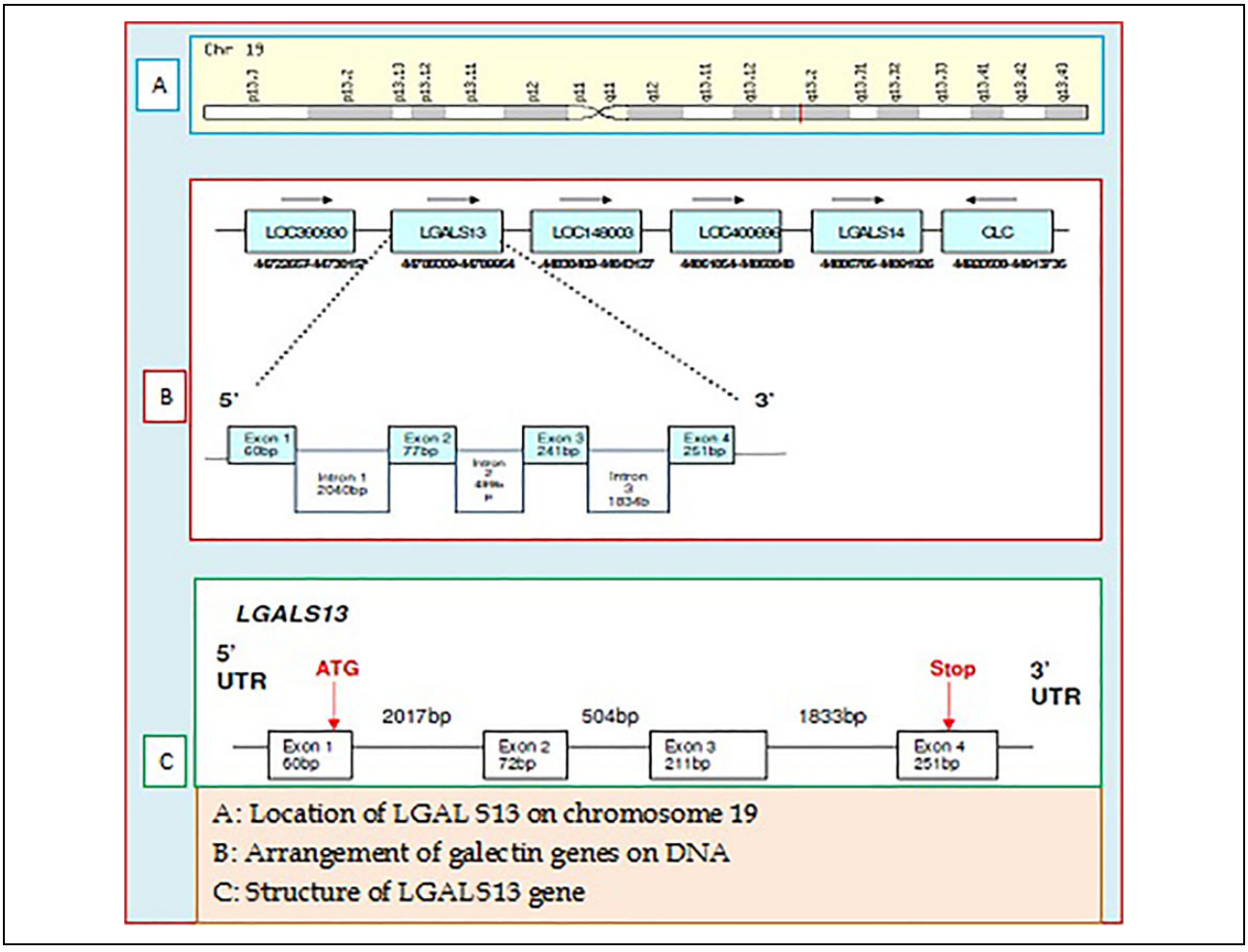

Figure 1. Location, arrangement, and structure of LGALS13 gene.[53]

and also in few pathological conditions of liver adenocarcinoma, neurogenic tumor, and malignant melanoma.[46]

Physical and chemical characteristics of PP13

Human PP13 is the member of the β-galactoside binding soluble-type galectin super family and is a relatively small protein with a molecular weight of 32 kDa consisting of 139 amino acid residues. It is a homodimer stabilized by disulfide bonds. Each polypeptide chain molecular weight is 16 kDa.[47] The primary structure of PP13 has 69% homology to human eosinophil Charcot–Leyden crystal structure (galectin 10).[48] The secondary structure is similar to prototype galectins such as galectin 7 and galectin 10 or Charcot–Leyden crystal protein.[49,50] The C-terminal CRD is of chimera-type galectin (galectin 3).[51]

The typical structural alignment of these proteins showed five-stranded (F1–F5) and six-stranded (S1–S6 a/S6b) β-sheets stabilized by one or two alpha-helices at their ends (Figure 3). The structural variation in sequence of primary structure of PP13 and galectin 10 is evident but the secondary structure is found to be identical except for F1 beta-sheet which is longer by one residue in case of PP13. From repository, the model was available from Brookhaven protein data bank source with account number IF87.[46] The dimeric nature of PP13 consists of two structurally similar disulfide bonds between four cysteine residues (Cys19, Cys92, Cys136, and Cys138) present on the surface of PP13. The alignment of galectin CRD motif showed highly conserved sequences of 13 residues, of which 8 residues (arginine 53 (Arg53), asparagine 65 (Asn65), tryptophan 72 (Trp72), Glu75, arginine 55 (Arg55), His57, Val63, and Thr77) play an important role in sugar binding. From these eight residues, four residues are of identical type (Val63, Asn65, Trp72, and Glu75) and three are conservatively substituted (Arg53, Arg55, and His57) in PP13.[46]

With respect to substrate automatic docking with CRD region particularly on the concave face of S4–S6 β-sheets, several carbohydrates as ligand to protein conformations were analyzed. They are *N*-acetyllactosamine, lactose, mannose, *N*-acetylgalactosamine, galactose, and so on, from which *N*-acetyllactosamine has more binding energy (−26.9 kcal/mole) due to high van der Waals forces and

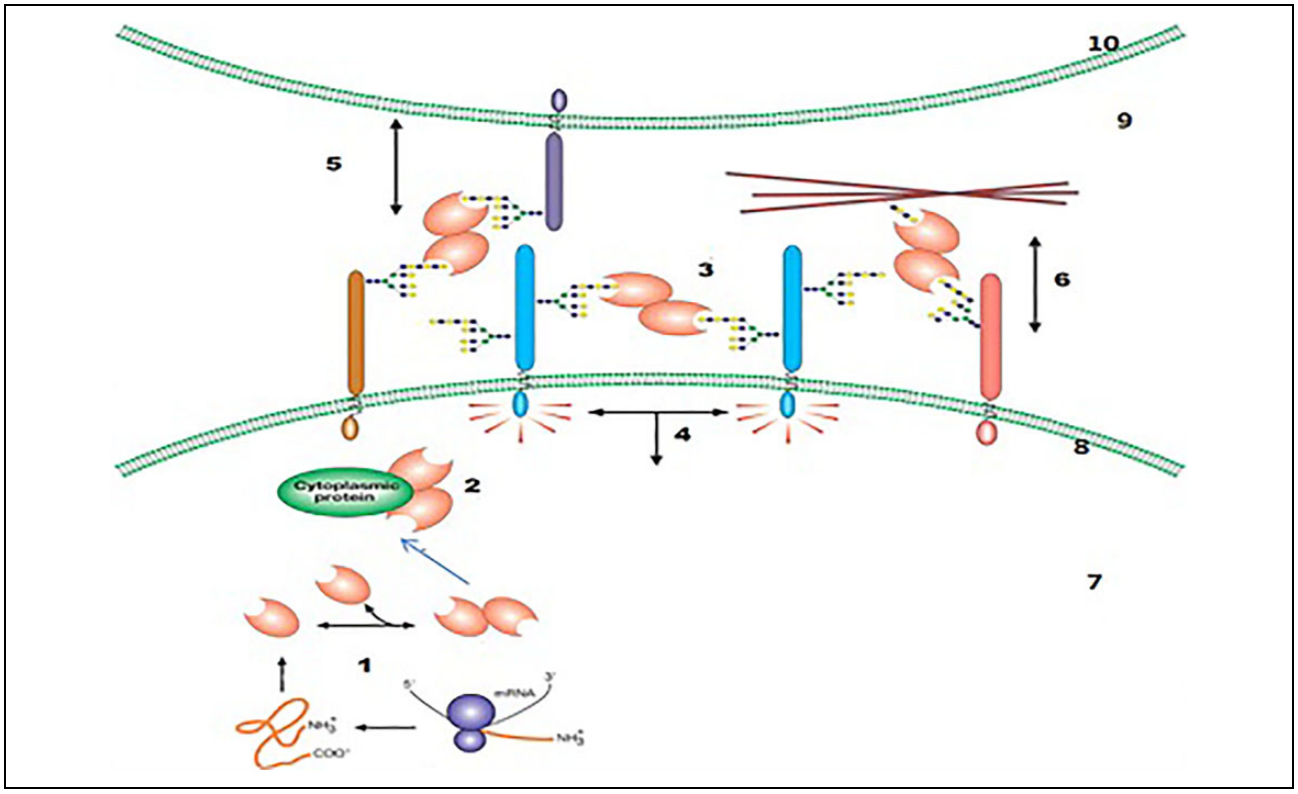

Figure 2. Expression, secretion, and functions of PP13. (1) Bio-synthesis: PP13 transcripts are translated on the free ribosomes in the cytoplasm of syncytiotrophoblast and dimerized as prototype PP13 with formation of carbohydrate recognition domain. (2) Intracellular protein–protein interactions: Actin cytoskeleton and motor proteins in the cytoplasm interaction drive the translocation of PP13 to the apical membrane of the syncytiotrophoblast and extracellular space. (3) Interaction with cell surface glycans and lattice formation: PP13 cross-links with glycoconjugates on cell surfaces and forms galectin–glycan lattice. (4) Cross-linking and signaling. (5) Cell–cell interactions: The cross-linked dimeric PP13 and lattice lead to intracellular signaling cascade to promote cell–cell interactions. (6) Cell–matrix interactions: Binding to β-galactoside residues of extracellular matrix proteins generates response to PP13 functions. (7) Cytoplasm of syncytiotrophoblast. (8) Apical membrane of syncytiotrophoblast. (9) Extracellular matrix. (10) Decidual membrane. PP13: placental protein 13.[28]

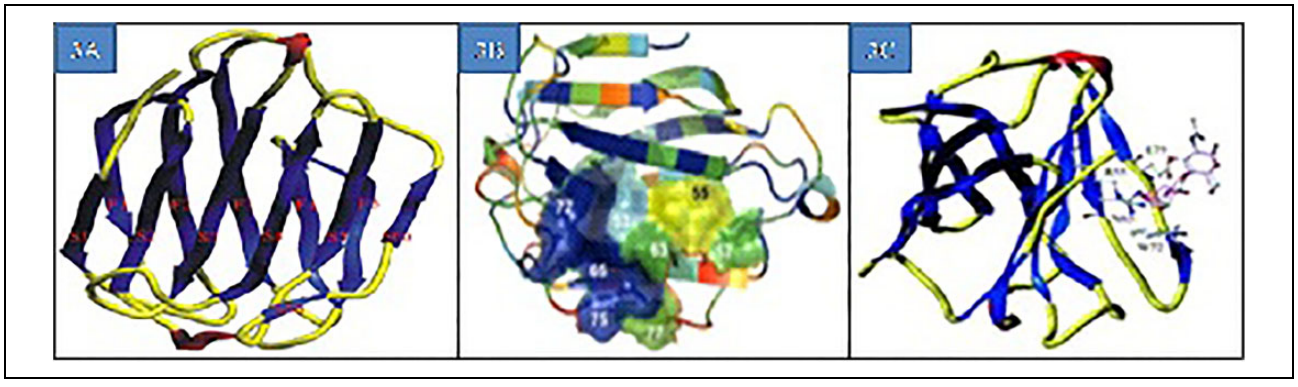

Figure 3. (a) PP13 "jelly-roll" fold structure indicating five- and six-stranded β-sheets linked by two alpha helices. (b) Eight amino acid residues on the carbohydrate recognition domain of PP13. (c) Stereoscopic view of PP13 molecule with bound *N*-acetyllactosamine to the residues in the carbohydrate recognition domain (A455, Asn65, and Gln75) and the tryptophan ring (Trp72) playing a key role in stacking interactions[46] PP13: placental protein 13; Asn65: asparagine 65; Gln75: glutamine 75; Trp72: tryptophan 72.

strong stacking interaction. This interaction has affinity to highly conserved Trp72 residue and strong hydrogen bond with conserved Arg55, Asn65, and glutamine 75 (Gln75) residues in CRD of PP13. This results in PP13 specificity for the sugar provides the cellular selection of binding molecules and its orientation toward binding.

Computational analysis indicated that the positions of serine and tyrosine phosphorylation sites are serine 48, tyrosine 41, and tyrosine 80 close to the highly conserved CRD.[46]

PP13 is a glycoprotein with a 0.6% carbohydrate content and has the electrophoretic mobility same as that of albumin; isoelectric point (PI) is in the range of 4.7–4.8 and sedimentation coefficient is 3.1 svedberg or S value. The primary structure of PP13 has two linear polypeptide chains. Each chain has methionine at the C-terminal and asparagine at the amino terminal.[30] Annexin II and β/γ-actin were identified as ligands for PP13 by the mass spectrometry; hence, these proteins are considered to play a key role in placentation and maternal artery remodeling, respectively.[52]

Structure of LGALS13 *gene and its mutations*

The *LGALS13* gene encoding for PP13 is expressed by the placental syncytiotrophoblast on chromosome 19 (19q13.1). *LGALS13* gene and its transcribing unit codes for PP13 at 5′ end that has promoter region followed by comprising four exons designated as E1–E4, which consist of base pairs in E1(60 bp), E2 (72 bp), E3 (211 bp), and E4 (251 bp) spaced by introns. Intronic regions vary between 499 bp and 1834 bp in length. Exon 4 and part of exon 3 of *LGALS13* gene exclusively code for the entire CRD.[53]

Many studies reported that downregulated placental expression of *LGALS13* is due to mutations in the gene, characterized by single nucleotide polymorphism (SNP) and single nucleotide deletion (delT221 mutation) in exon 3, mutations of the exon–intron boundaries (Dex-2 mutation), and an SNP in promoter region. The DNA variants result in the expression of truncated protein which cannot bind carbohydrates. The implication of altered, misfolded, and nonfunctional protein may be associated with a variety of obstetrical syndromes such as preeclampsia where the normal implantation and placentation are generally disrupted.

In support of this genetic evidence, decreased PP13 levels and its messenger RNA (mRNA) expression of *LGALS13* in the first trimester of pregnancy,[36,54–57] SNP (221delT) with low PP13 level is seen in adverse pregnancy conditions[58–60] and intronic polymorphisms are associated with PP13 level and impaired of lysophospholipase activity.[52,59–61] The promoter variants with SNPs were found in women with increased risk to develop preeclampsia.[62–64] Transcription factor activating enhancer binding protein 2 alpha (TFAP2A) is a protein encoded by human TFAP2A gene. It binds to specific DNA sequence and recruits transcription machinery. It specifically binds to G regions at −98 position than to A in the promoter region and induces promoter expression as revealed by PP13 promoter reporter expression studies after transfecting BeWo cells with PP13 having "A" or "C" in the −98 promoter position.[65]

Decreased placental expression of glial cell missing-1 and estrogen-related receptor γ genes, encoding transcription factors, regulates *LGALS13* expression in the syncytiotrophoblast which is associated with trophoblast fusion and syncytium formation.[18] According to the available literature, the following few studies clearly demonstrate that decreased PP13 level or impairment in expression and its possible genetic causes are seen in women with preterm preeclampsia. Ongoing research to determine whether the presence of polymorphic PP13 variants may serve to improve our understanding of the underlying pathophysiology is of paramount importance.

The mutation in the promoter region and the exons of *LGALS13* gene is responsible for lower expression of PP13 mRNA and the low levels of plasma PP13 in the first trimester. Therefore, genetic analysis of *LGALS13* and plasma PP13 level is one among the several biomarkers studied to understand preeclampsia and its complications at early stage to facilitate antenatal checkup.

Quantification of PP13

The quantity of PP13 from serum, plasma, tissue fluids, and placental tissue was carried out by employing immune-based technique—enzyme-linked immunosorbent assay (ELISA) which is currently widely employed procedure for PP13 assay from biological samples. The results published from this assay method in several research studies generate varied information in different trimesters of normotensive pregnancies and subjects likely to develop preeclampsia and eclampsia. Measurement of PP13 is not a routine investigation in clinical medicine but gained much attention in pregnancy-related research. In many studies, quantification of PP13 in biological sample was done either by ELISA[19–22,66–76] or by dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) methods.[77–86]

The underlying principle of ELISA for the estimation of PP13 is an enzyme-linked antibody sandwich method. The test principle is that the microtiter plate is pre-coated with an antibody specific to PP13. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to PP13. Avidin conjugated to horseradish peroxidase is added to each microplate well and incubated. After the addition of tetramethylbenzidine substrate solution, only those wells that contain PP13, biotin-conjugated antibody, and enzyme-conjugated avidin will exhibit a change in color. The enzyme–substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured at a wavelength of 450 nm. The concentration of PP13 in the samples is then determined by comparing the absorbance of the samples with the standard curve. DELFIA technique is also used to estimate PP13 in many research contexts. The unit for representing the quantification is picogram per milliliter or nanogram per milligram of tissue. It is reported that the normal term placenta yields

nearly 3.7 mg of PP13.[28] The advantage of ELISA method over DELFIA is that the reducing agent dithiothreitol in the sample dilution buffer of the ELISA keeps the PP13 in monomer form which prevents its affinity to the sugar residues of various blood proteins especially of the erythrocytes.

Functions of PP13

Placental implantation

Placental implantation involves attachment of the blastocyst to the endometrial epithelium and subsequent invasion in order to form a functional placenta. It occurs through the trophoblast, an outer layer of blastocyst, that subsequently develops into syncytiotrophoblast, which is involved in implantation and placentation.[87] It is known that placentation also involves PP13, a member of group of pregnancy-related proteins. It is highly expressed in placenta at maternal–fetal interface and may play an important role in the maintenance of pregnancy (e.g. embryo implantation, maternal–fetal immune tolerance, placentation, and vascular remodeling). The fact that structural and functional characteristics of this protein and its role are important in placental development and regulation pathways is receiving increased interest in recent times.[88] The carbohydrate binding specificities were extensively studied by numerous extracellular methods such as solid phase assays,[89,90] surface plasmon resonance,[91] fluorescence polarization,[92] frontal affinity chromatography,[93] and isothermal calorimetry.[94]

β-human chorionic gonadotropin induced trophinin is an intrinsic membrane protein expressed on the apical membrane of the trophectoderm cells which is involved in adhesion of blastocyst to the endometrial epithelium during implantation process.[95,96] In addition to this, binding of PP13 to the glycosylated receptor is also important for adhesion and signaling in placentation. Based on sugar binding assay results, it has been found that PP13 has the strongest binding affinity to the *N*-acetyllactosamine, mannose, and *N*-acetylglucosamine residues of carbohydrate moiety in glycoproteins and glycolipids present on placenta.[97] An automatic docking study done using FlexX module of SYBYL determined that the carbohydrate molecules such as *N*-acetyllactosamine, lactose, mannose, *N*-acetylgalactosamine, and galactose are located at different ligand positions at the CRD.[98–100] Another study has also reported that all the carbohydrate conformations were characterized by their binding energies[101] and PP13 has the highest van der Waals interaction with *N*-acetyllactosamine (−26.9 kcal/mol).

A strong stacking interaction with the aromatic ring of Trp72 and three strong hydrogen bond interactions (<2.5 Å) with Arg55, Asn65, and Gln75 residues in CRD of PP13 were detected. The conserved Arg53 and Arg55 in the CRD play a critical role in the interactions between PP13 and the lactose moiety. Mannose has the lowest van der Waal interaction with the PP13 but no hydrogen bond interactions were reported (Table 1).[46] Sugar binding assays to determine the affinity property found that PP13 exists as homodimer stabilized by disulfide, a bond linkage, which is favorable for hemagglutination in terms of lectin activity. Reducing agents diminish dimerization and reduce sugar binding affinity and hemagglutination activity. This unique property of PP13 affects its biological activity in the placenta.[102]

Table 1. Van der Waals energies of PP13-ligand complexes.

Ligand	van der Waals energy (kcal/mol)	Number of hydrogen bonds	Amino acid residues involved in hydrogen bonding
N-acetyl-D-lactose amine	−765.2	3	Arg55, Asn65, Gln75
Lactose	−759.4	2	Arg55(2)
N-acetyl-D-galactosamine	−751.9	2	Arg55(2)
Galactose	−751.4	3	Arg53(2), Asn65
Mannose	−741.5	0	—

PP13: placental protein 13; Arg55: arginine 55; Asn65: asparagine 65; Gln75: glutamine 75; Arg53: arginine 53.

In vivo, placentally expressed purified PP13 was found to be phosphorylated. Experiments done with placental PP13/galectin 13 showed that it contains phosphorylation sites. Computational analysis indicated sites for serine/tyrosine kinase phosphorylation at positioning Ser48 (44–57) and Tyr41 (37–45) and Tyr80 (76–84) close to CRD domain. The carbohydrate binding affinity was observed to be the same in PP13-B (placentally expressed phosphorylated) and PP13-R (bacterially expressed non-phosphorylated). The only possible linkage of phosphorylation is to lysophospholipase activity. Since limited information is available on phosphorylation and functional properties of PP13, it is necessary to establish the exact role of phosphorylated PP13.[103]

Regarding the hemagglutination process, several research reports (Table 2) showed that PP13 has the tendency to bind to β-galactosides located on the terminal positions of ABO blood group antigens. Immunoassay staining intensity of placental tissue showed variable PP13 binding to RBC antigens of respective blood groups A, B, and O.[34] Research reports pertaining to relationship between different blood groups and preeclampsia risk indicated mixed results regarding the effect of blood groups on preeclampsia severity. A review of a few studies looking at this subject suggests higher risk of preeclampsia with A blood group,[104,105] with O blood group,[106,107] and with AB blood group.[34,105,108–113] Yet a few more studies suggested no relationship between them.[114–118] A limited number of studies explored the association of different blood groups with decreased bioavailability of PP13.[34,113,119] Further research and large cohort studies are required to

Table 2. Research studies showing association of blood groups A, B, and O with preeclampsia.

S.No.	Author	Year	Study design	Results
1	May	1973	Case control	Association of blood group A with preeclampsia than O blood group
2	Scott and Beer	1976	Case control	No association between ABO blood group and preeclampsia risk
3	Amin et al.	1989	Case control	Blood group O as the risk factor for preeclampsia
4	Spinillo et al.	1995	Case control	Maternal AB blood group associated with increased risk of severe preeclampsia
5	Witsenburg et al.	2005	Case control	ABO blood group never observed as risk in pregnancy complications (preeclampsia, HELLP syndrome, and PIH).
6	Clark and Wu	2008	Prospective cohort	No effect of ABO blood type on the risk of preeclampsia
7	Hiltunen et al.	2009	Nested case control	Preeclampsia risk associated with AB blood group
8	Than et al.	2011	Case control	Maternal blood group AB contributes to less PP13 bioavailability and increased risk of preeclampsia
9	Lee et al.	2012	Cohort	AB blood group associated with high risk of developing preeclampsia than O blood group
10	Phaloprakarn and Tangjitgamol	2013	Case control	Blood groups A and AB were associated with increased risk for preeclampsia
11	Hentschke et al.	2014	Case control	No association between blood groups and preeclampsia
12	Seyfizadeh et al.	2015	Case control	ABO blood group associated with unfavorable outcomes of pregnancy
13	Manjunatha and Anita	2015	Cross-sectional	AB blood group has highest preeclampsia risk
14	Avci et al.	2016	Case control	AB blood group with decreased availability of PP13 associated with higher risk of preeclampsia
15	Elmugabil et al.	2016	Case control	Blood group O with higher risk for preeclampsia
16	Reisig et al.	2016	Case control	Blood group A with increased risk of preeclampsia
17	Mital et al.	2016	Case control	AB blood group with higher risk of preeclampsia.
18	Aghasadeghi and Saadat	2017	Case control	No association between ABO blood groups and preeclampsia risk
19	Beyazit et al.	2017	Case control	No association between blood groups and preeclampsia

PP13: placental protein 13. PIH: pregnancy induced hypertension.

investigate the effect of blood groups on the availability of PP13 and its association with preeclampsia.

Immunological function

Maternal immunological response is essential for the establishment, maintenance, and completion of a healthy successful pregnancy. The human semi-allogeneic fetus is not rejected by the maternal immune system, despite expressing paternal antigens due to many fetal, maternal, and placental mechanisms that have been implicated in aiding fetal tissues in escaping maternal immune attacks. The immunological barrier (placenta and maternal decidua) plays a crucial role in acceptance of the fetus and making the decidua more receptive for the invading fetus.

Of the numerous mechanisms involved, uterine natural killer cells (regulate the trophoblast invasion through secretion of angiogenic growth factors),[120] cytokines and chemokines (interleukin-8 and interferon inducible protein-10),[121] dendritic cells,[122] macrophages,[123] the complement system,[124] Toll-like receptors,[125] decidualized endometrium secretion,[126] the trophoblast human leucocyte antigen (HLA) expression pattern,[127] Fas ligand,[128] indoleamine 2,3-dioxygenase,[129] B7 family,[130] T helper cells,[131] and T regulatory cells[132] are all implicated in modulating immune responses for an effective implantation. The inadequate maternal–fetal immune tolerance is one of the proposed mechanisms leading to preeclampsia. Breakdown of maternal–fetal tolerance also leads to other pregnancy-specific autoimmune diseases, bleeding complications during the first trimester, pregnancy-induced hypertension, and preterm or recurrent miscarriages.[133]

So apart from the abovementioned mechanisms, human placenta-specific galectins expressed by the syncytiotrophoblast at the maternal–fetal interface are key regulator proteins of the immune responses. They confer immune tolerance to the semi-allogeneic fetus by bringing about the apoptosis of maternal T lymphocytes to sustain placentation.[134,135] PP13 drains through the decidual veins in the form of exosomes/microvesicles into the decidua where it attracts and activates maternal immune cells, diverting them away from maternal spiral arteries to facilitate uninterrupted spiral artery remodeling. PP13 plays a unique role in early pregnancy by forming decidual crystal-like aggregates within decidual zones of necrosis. These zones are associated with necrotic and apoptotic immune cells which are CD45RO memory T cells, CD68 + macrophages, and CD57 + large granular lymphocytes. The decidual zones of necrosis peak at 7–8 weeks of gestation when placental circulation is not yet established and diminish after the

completion of spiral artery remodeling at about 10–14 weeks of gestation. The diversion of these immune cells facilitates uninterrupted trophoblast invasion and remodeling of the maternal spiral arterioles.[17]

As the demands of the growing fetus for oxygen and nutrients increase as the pregnancy progresses, the transformation of the maternal vasculature becomes critical. Impaired spiral artery remodeling may be linked to an immune maladaptation syndrome, preeclampsia followed by failure of the fetus to reach its optimal growth, and intrauterine growth retardation (IUGR).[136–139] Among the studied recombinant galectins, the apoptotic effects of PP13 on freshly isolated activated CD3 + T cells were stronger when compared to galectin 1. The PP13/galectin 13 functional role with respect to T cell apoptosis is similar to that of the mechanism behind T cell apoptosis caused by galectin 1.[28]

However, the similar effect was not found with truncated PP13 due to lack of CRD function and T cell apoptotic activity. Hence, structural integrity of PP13 is crucial in sugar binding and the regulation of cell–matrix interactions for successful human placentation. Thus, inadequate placental expression and structural derangement with altered function of PP13 result in adverse conditions during pregnancy and poor obstetric outcome.[36] The involvement of PP13 as pro-inflammatory function demonstrated in in vitro study where PP13 triggered the secretion of cytokines and chemokines into the culture medium from mononuclear cells isolated from peripheral blood of pregnant women. Thus, varied action of PP13 with respect to apoptotic and pro-inflammatory function studied in in vitro experiments.[28]

Regulation of blood pressure

The role of PP13 in embryo implantation in maternal–fetal interface has been reported. But animal experiments and studies in humans regarding the role of PP13 in blood pressure regulation are lacking. Nonetheless, there are studies which suggest that PP13 contributes to vasodilation and remodeling of uteroplacental vasculature and improves pregnancy outcome.[140–142] The exact molecular mechanism of vasodilation and the unidentified responsive element that influences endothelial nitric oxide synthase and cyclooxygenase enzymes in preeclampsia model are yet to be elucidated even though prostaglandins do play an important role in vascular remodeling during early placentation.[142]

Introduction of PP13 to cultured trophoblasts elicited depolarization of the trophoblast membrane by calcium ions leading to liberation of linoleic and arachidonic acids from the trophoblast membrane lipids and this leads to increased amount of prostacyclin and thromboxane synthesis.[50] The in vivo vasodilatory effects of PP13 in preeclamptic animal models and the levels of PP13 correlated with nitric oxide and prostaglandin levels. This needs to be further investigated in order to evaluate the potential therapeutic use of PP13 in humans for improving blood flow and pregnancy outcome in hypertensive disorders of pregnancy or preeclampsia.

Recombinant PP13

Recombinant PP13 (PP13-R) was genetically engineered and produced by Hy-laboratories (Hylabs Rehovot, Israel). The basis for PP13 sequence construct has been validated by Sanger sequencing information published by National Centre for Biotechnology Information, USA. The same sequence constructs are expressed in transfected *Escherichia coli*. The expressed protein was harvested and affinity purified, verified by SDS PAGE, HPLC, and immunoblot using PP13-specific monoclonal antibodies. Several in vivo and in vitro extensive studies explored the functional aspects of recombinant PP13 in biomedical research.

In a protein conformation study, PP13-R was reported as a homodimer stabilized by disulfide bonds possessing binding strength to different sugars in nonreducing conditions. However, the reducing conditions favorable for the monomeric form lack binding ability to sugars.[58]

In vitro study on hemagglutination of human erythrocytes described the binding affinities of common recombinant PP13 (PP13-R) and truncated recombinant PP13 (TrPP13). PP13-R has the strongest binding affinity to the β-galactosides on the erythrocyte surface of the blood group AB and weakest affinity to erythrocytes of blood group B. However, this is not exhibited by TrPP13 due to altered CRD. This provides evidence that conserved CRD is crucial to exhibit lectin activity.[28] The major in vitro study information is that the immune response of recombinant galectin was studied and noted that among the galectins, PP13-R had the strongest apoptosis-inducing effect on the freshly isolated maternal T cells. This observation laid the strong foundation to understand the central role of PP13 in maintaining immune tolerance at the maternal–fetal interface.[36]

In an experimental animal model, the role of CRD of PP13 was elucidated in pregnancy by comparing the wild-type recombinant PP13 and truncated variant on administering both the variants to pregnant rats. The significance of wild-type PP13 in regulating blood pressure and expanding uteroplacental vasculature was noted. This suggests that PP13 may have a potential therapeutic role during pregnancy risk.[65] Phosphorylation of the protein side chains of PP13 expressed and purified from placenta indicated the specific biological function and necessitates further research in order to understand the role that the phosphorylated protein plays and its mechanism. The inherent biological property restored on wild-type PP13 and PP13-R studied and compared by phosphorus-31 nuclear magnetic resonance analysis and found that both variants exhibited weak endogenous lysophospholipase activity. The affinity purified wild-type PP13 and PP13-R when incubating with

protein extracts from human placenta or WRL-68 fetal hepatic cell lines identified annexin-II and β/γ-actin as specifically bound proteins for PP13.[101]

The role of PP13-R on the cardiovascular system in gravid and nongravid rodents showed, for the first time, the influence of PP13 on vasodilation, uterine artery remodeling, and in improving uteroplacental blood flow as well as adaptation of the maternal vasculature to pregnancy. The same author also studied the role of PP13-R on pregnant rats in lowering blood pressure, venous remodeling, and improving fetal growth.[140] Administration of truncated PP13 variant resulted only in a hypotensive effect with loss of venous remodeling and increase in placenta and pup weights.[79] In a recent animal study, the role of PP13-R in endothelial signaling pathways of vasodilation of resistance arteries from pregnant and nonpregnant rats was studied and concluded that PP13 may become useful therapeutically in improving blood flow and pregnancy outcomes in hypertensive pregnancies.[142]

Screening performance

Preeclampsia is one of the most common and life-threatening disorders of pregnancy affecting a total of 8.5 million women worldwide. Preeclampsia is responsible for 18% of maternal deaths and up to 40% of fetal mortality. Despite extensive research, preeclampsia still lacks a safe, cost-effective, reliable, early means of diagnosis, or prediction. PP13/galectin 13 produced by the syncytiotrophoblast during pregnancy is involved in normal placentation. In normal pregnancies, serum levels of PP13 slowly rise with gestational age. Several studies have reported that decreased serum levels of PP13 in the first trimester increase the risk of subsequently developing preeclampsia. Measurement of PP13 levels as a first trimester screening marker for preeclampsia may provide an opportunity for identification of women destined to develop early-onset preeclampsia.

Romero et al.[69] studied a cohort of 300 patients, of which 50 developed preeclampsia. Serum PP13 concentration in the first trimester was significantly lower in patients who developed preterm and early-onset preeclampsia than in those with normal pregnancies; and at 80% specificity, a cutoff of 0.39 multiples of median (MoMs) had a sensitivity of 100% for early-onset preeclampsia and 85% for preterm preeclampsia. Based on these results, it was concluded that the first trimester maternal serum PP13 concentrations can be used in the risk assessment for preeclampsia.

Gonen et al.[15] studied a cohort of 1366 pregnant women and subsequently 20 were diagnosed with preeclampsia. At 6–10 gestational weeks, PP13 levels were significantly lower among the preeclampsia group with a median of 0.28 MoM (95% confidence interval (CI) 0.15–0.39, $p < 0.004$). Using a cutoff of 0.40 MoM, the sensitivity was 80%, a false positive rate (FPR) was 20%, and odd ratio was 16.0 (95% CI 18.2–169.2). This study reported that PP13 in the first trimester alone or in combination with the slope between the first and second trimesters may be a promising marker for assessing the risk of preeclampsia.

Nicolaides et al.[66] studied PP13 as a biochemical marker for the early onset of preeclampsia at 11 + 0 to 13 + 6 weeks of gestation. At an FPR of 10%, PP13 showed a prediction rate of 80% as a single biochemical marker. In combination with Doppler ultrasound PI, the prediction rate increased to 90%.

Spencer et al.[68] conducted a nested case-control study to evaluate whether the measurement of maternal serum PP13 and pregnancy associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks of gestation alone or in combination with the second trimester uterine artery pulsatility measured by Doppler velocimetry is useful in predicting those women who will develop preeclampsia. There were 446 controls and 44 cases with early preeclampsia where delivery was induced prior to 35 weeks. In addition, there were 44 cases with preeclampsia in which delivery was not induced before term. Median PP13 levels for controls, all cases, and early preeclampsia cases were 176.9, 121.9, and 111.7 pg/mL, with MoMs of 1.00, 0.69, and 0.63, respectively ($p < 0.001$). The first trimester PP13 levels may be useful in predicting preeclampsia and early preeclampsia, and the accuracy of the method increases when coupled with the second trimester Doppler PI measurement. Further studies are required to establish the real value of PP13 in the first trimester screening for preeclampsia.

Plasma PP13 has also been used in combination with urinary glycosaminoglycans/proteoglycans as early markers for preeclampsia by De Muro et al.[20] A total of 62 women were enrolled in the study, of which 24 presented complications such as preeclampsia, proteinuria, and hypertension during pregnancy. Plasma levels of PP13 were significantly reduced in the group of women who went on to develop complications compared with controls ($p = 0.022$). The reduced plasma levels of PP13 and the alteration of the relative content of urinary glycosaminoglycans and proteoglycans observed in their study could be a promising tool for the prediction of preeclampsia in an early stage of pregnancy.

MoslemiZadeh et al.[75] conducted a prospective nested case-control study that recruited 1500 pregnant women and 100 women developed preeclampsia and represented the case group. Of 100 women with preeclampsia, 66 cases have mild preeclampsia, while 34 cases have severe preeclampsia. Serum PP13 levels along with PAPP-A were measured in the first and second trimesters and were significantly lowered in women who developed preeclampsia ($p < 0.001$). The cumulative value of all the four variables with cut-off points of 238.5 has sensitivity and specificity of 91.0% and area under curve of 0.968. The study concluded that measuring PP13 with PAPP-A in both trimesters of pregnancy is advantageous for the prediction of the incidence of preeclampsia.

Wortelboer et al.[81] conducted a nested case-control study to investigate the predictive value of PP13 along with other biochemical markers such as Pregnancy associated plasma protein A (PAPP-A), β-human chorionic gonadotropin (β-hCG) and Placental growth factor(PlGF) and desintegrin ADAM 12 in the first trimester of pregnancy. PP13 and PlGF were reduced in women with preeclampsia, with median of 0.68 and 0.73 MoM, respectively ($p <$ 0.0001 for both). This study demonstrates that PP13 and PlGF in the first trimester might be promising markers in risk assessment for early preeclampsia/HELLP syndrome.

Odibo et al.[73] conducted a prospective cohort study of pregnant women followed from the first trimester to delivery as part of a first trimester screening study for adverse pregnancy outcomes. This study was conducted on 477 women of whom 42 women were diagnosed with preeclampsia. For a fixed FPR of 20%, PP13, PAPP-A, and mean uterine artery pulsatility index identified 49, 58, and 62%, respectively, of women who developed any form of preeclampsia. PP13 was best in predicting early onset preeclampsia with a sensitivity of 79% at an FPR of 20% and can be considered as individual predictors of women at risk to develop preeclampsia.

Svirsky et al.[76] conducted a cohort study to determine the first trimester maternal serum PP13 in singleton versus twins with and without severe preeclampsia. In twins, the median was 1.74 MoM ($n = 76$) versus 1.74 in unaffected twins ($p = 0.10$) and 2.26 ($n = 6$) for mild preeclampsia ($p = 0.30$). Among singletons with severe preeclampsia, the median was 0.44 MoM ($n = 26$, $p < 0.0001$), and for mild preeclampsia 0.62 ($n = 17$, $p < 0.001$). Based on the results, they concluded that PP13 was higher in twins than singletons corresponding to larger placental mass.

Beljan et al.[22] conducted a prospective study to detect whether the first trimester serum PP13 and copeptin can predict preeclampsia in advanced age nulliparous women. This study confirmed a significant contribution for the combination of two biomarkers for the prediction of preeclampsia ($p = 0.007$). Decreased level of PP13 and an increased level of copeptin can predict preeclampsia with a sensitivity of 66.7% and a specificity of 97.3%, with an FPR of 2.7%.

El Sherbiny et al.[74] conducted a cohort study on 100 women to assess the value of PP13 as an early marker for screening of preeclampsia and to correlate with the PP13 mRNA. The maternal serum PP13 level in the preeclamptic group was (157.9 $\pm$ 45.5 pg/mL), which is significantly lower than that of the control group (225 $\pm$ 67.3 pg/mL), with a highly statistically significant difference ($p < 0.0001$). The frequency of maternal PP13 was also lower in the preeclamptic group (28%) compared to that in the control group (76%), with a highly statistically significant difference ($p < 0.0001$). This study concluded that serum PP13 assay and PP13 mRNA expression are reliable markers for early detection of preeclampsia and was recommended doing it as a routine investigation during the first trimester.

Khalil et al.[71] conducted a nested case-control study to evaluate whether the first trimester maternal serum PP13 can predict preeclampsia in women with a priori high risk. Women who developed preeclampsia had significantly lower ($p < 0.001$) PP13 MoMs compared with controls. PP13 MoMs for controls and preeclampsia cases were 1.0 (range 0.0–10.0) and 0.4 (range 0.0–7.0), respectively ($p <$ 0.001). At an MoM cutoff of 0.53, for an FPR of 10%, sensitivity was 50% for preeclampsia at term (>37 weeks), 62% for preterm preeclampsia (<37 weeks), and 71% for early onset preeclampsia (< 34 weeks). Based on these results, the first trimester PP13 can predict preeclampsia in women at increased priori risk and predicts early-onset better than late-onset disease.

Chafetz et al.[67] performed a prospective, nested case-control study to evaluate the first trimester serum PP13 as a screening test for preeclampsia and intrauterine growth restriction. The median first trimester PP13 level was 132.5 pg/mL in the control subjects ($n = 290$). Median PP13 levels were significantly lower among women who had preeclampsia (27.2 pg/mL; $p < 0.001$), IUGR (86.6 pg/mL; $p < 0.001$), and preterm delivery (84.9 pg/mL; $p = 0.007$). When PP13 was expressed as multiples of the gestational age-specific medians among the control subjects, the MoMs were 0.2 for preeclampsia, 0.6 for IUGR, and 0.6 for preterm delivery ($p < 0.001$ for each disorder compared with subjects). Receiver operating characteristic analysis yielded areas under curve of 0.91, 0.65, and 0.60 for preeclampsia, IUGR, and preterm delivery, respectively. At a 90% specificity rate, the corresponding sensitivities were 79, 33, and 28%, respectively. This study concluded that maternal PP13 levels in the first trimester are a promising diagnostic tool for the prediction of preeclampsia with high sensitivity and specificity.

Huppertz et al.[70] conducted a prospective longitudinal study with 41 normal pregnant women, 18 cases with preterm delivery or cervix insufficiency, and 4 with developing late-onset preeclampsia. Six hundred and sixty-six blood samples were obtained every 2–4 weeks starting from 5 weeks to 8 weeks of gestation (10–12 samples/patient) and tested for serum PP13. In normal pregnant women delivering at term, median maternal serum PP13 levels were increasing from 166 pg/mL to 202 pg/mL and 382 pg/mL in the first, second, and third trimesters, respectively. Preeclamptic women had significantly reduced PP13 levels in the first trimester (MoM of 0.14 at 7–8 weeks; $p = 0.005$ compared to normal). PP13 in the third trimester was significantly higher compared to normal at 35–36 weeks with PP13 MoM of 1.79. This preliminary study indicates that low levels of PP13 in early pregnancy indentify at risk pregnancies, whereas high levels precede the syndrome in late pregnancy and suggest syncytiotrophoblast necrosis.

A large number of cohort studies are required to determine the study replicability and to establish PP13 measurements as an early screening indicator in larger population. However, the exact reason for lower level of PP13 bioavailability in the first trimester of the susceptible subjects likely to develop preeclampsia is still unknown. Despite considerable amount of research, development of sensitive, stable, reliable, and cost-effective biochemical analytes is of great importance.

Future perspectives

Reliable early screening for preeclampsia is one of the most important challenges in prenatal care, since preeclampsia is a serious complication and an important cause of maternal and perinatal morbidity and mortality with a prevalence of 2–8%[2] resulting in about 50,000–60,000 deaths annually worldwide.[143] It is responsible for 18% of maternal deaths and up to 40% of fetal mortality.[6] According to World Health Organization, the mortality rate of preeclampsia in developing countries is seven times higher than developed countries.[144]

PP13 is produced by the syncytiotrophoblast throughout pregnancy. The significantly reduced levels in the first trimester make it a promising tool for identifying women at increased risk of preeclampsia and scope for research to find the exact cause for low levels of PP13 at the first trimester. However, whether it has a better predictive value when used alone or should be included in the highly sensitive panel of biomarkers needs to be determined by conducting larger prospective studies in different populations.

Data generated in animal studies suggest that PP13 has a beneficial effect in regulating blood pressure and the potential use of this galectin as a therapeutic drug. Administering PP13 to patients at risk for preeclampsia may be helpful in reducing the incidence of preeclampsia and in improving obstetrical outcomes.

An extensive research is necessary to study the effect of PP13 in arterial expansion and a thorough understanding of the mechanistic pathway in preeclamptic animal or human model. Hence, there is a scope to evaluate the efficacy of recombinant PP13 supplementation in alleviating pregnancy complications at conception. Thus, the outcome would gain momentum in advanced preeclampsia research through clinical trials. The PP13 structure has additional domains known to possess lysophospholipase activity and phosphorylation sites. The role of these functional domains in relation to normal pregnancy and in pregnancy complications still needs to be studied further.

Summary

The overall aim of this review article is to summarize the properties and biological functions of PP13 in normal pregnancy and in preeclampsia. PP13 is a 32-kDa protein localized in the brush border membrane of the syncytiotrophoblast lining at the common fetal–maternal blood spaces of the placenta. As a "prototype" galectin, it has a single sugar binding domain or CRD which emerges into the extracellular space and has lectin-like activity. The dimerized phosphorylated protein functions as a regulator of blood flow and blood pressure and has special immune functions at the fetal–maternal interface. PP13 induces different levels of hemagglutination, which depend on the type of blood group antigen, and it is involved in multistep process in pregnancy that supports trophoblast invasion as well as generation of maternal systemic endothelial effect. Further data are needed to establish whether dysregulation of PP13 levels in the maternal circulation in early pregnancy can serve as a reliable screening, predictive, and prognostic biomarker for preeclampsia.

Animal experimental results which found beneficial effects of PP13 in regulating blood pressure suggest the potential use of this galectin 13 as a therapeutic drug in preeclampsia. However, a large prospective and longitudinal studies are needed to confirm this observation. Although advanced research is focusing on molecular and epigenetic changes in preeclampsia, future study designs should first identify a highly sensitive and specific serum marker.

PP13 is known to be involved in different stages of pregnancy ranging from trophoblast invasion to vasodilation of the maternal vasculature needed for the increased blood flow to the fetus. Administration of recombinant PP13 in women with low serum levels may prevent impaired placental development in preeclampsia. Further research is required to completely understand the role of PP13 in the development of preeclampsia. The diagnosis of preeclampsia based on biomarkers might be useful in identifying patients at risk, monitoring disease progression to provide effective and timely interventions.

Conclusion

PP13, a placental galectin protein, is a homodimeric, phosphorylated protein localized in syncytiotrophoblast. By its CRD, it exerts erythrocytes agglutination or lectin activity through sugar binding capabilities. It is an immune regulatory, vasodilatory protein with weak lysophospholipase activity involved in placentation process. The low levels of blood PP13 acts as a new biological protein for individualized risk assessment and drug design target in management of preeclampsia. Preparation of monoclonal antibodies and recombinant PP13 helps in research for detection and replenishing the PP13 level in preeclampsia has attracted much attention.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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