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(54) Title: PLACENTAL PROTEIN 13

(57) Abrégé/Abstract:
The full amino acid and DNA sequences of placental protein 13 (PP13) are disclosed. Also described are various PP13 derived peptide fragments, and a recombinant method for the production of PP13. PP13 may be used in a screening and a diagnostic method for pregnancy-related complications.

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(57) Abstract

The full amino acid and DNA sequences of placental protein 13 (PP13) are disclosed. Also described are various PP13 derived peptide fragments, and a recombinant method for the production of PP13. PP13 may be used in a screening and a diagnostic method for pregnancy–related complications.

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PLACENTAL PROTEIN 13

FIELD OF THE INVENTION

The present invention relates to a placental protein and its uses.

BACKGROUND OF THE INVENTION

References referred to in the text by a number enclosed by parenthesis are listed at the end of the specification.

The goal of pregnancy management is the delivery of a mature, healthy infant, without encountering complications which can adversely affect the well being of both the mother and the newborn. A significant percentage of pregnancies are affected by various disorders. Among these complications are preterm labor and delivery, intrauterine growth retardation and preeclampsia. These conditions negatively impact the outcome of affected pregnancies, at enormous cost both to the patients as well as to the health system.

Placental Protein 13 (PP13) is a protein which was previously isolated from human placental tissue (U.S. 4,500,451 to Bohn, *et al.*.
The protein was characterized by the
following parameters: electrophoretic mobility, isoelectric point, sedimentation coefficient, molecular weight determined by ultracentrifugation, molecular weight determined by SDS-PAGE, extinction coefficient and carbohydrate content. The amino acid composition (residues per 100 residues) was determined but not the amino acid sequence.

PP13 was used to develop an assay for the early stage detection of three specific pregnancy-related disorders: intrauterine growth retardation, preeclampsia

and preterm delivery (U.S. 5,198,366 to Silberman). Both a radioimmunoassay (RIA) and an enzyme-linked immunoassay (ELISA) are disclosed using labeled PP13 and anti PP13 antiserum, respectively. No further properties of PP13 are disclosed in the Silberman patent.

BRIEF SUMMARY OF THE INVENTION

It is an object of the present invention to provide a pure PP13 protein.

It is a further object of the present invention to provide a DNA molecule encoding PP13.

It is a still further object of the invention to provide a recombinant method for producing PP13.

Additionally, it is an object of the present invention to provide a diagnostic assay based on PP13 for the early detection of pregnancy complications.

It is another object of the invention to provide immunogenic peptides derived from PP13 which can be used in such a diagnostic assay.

According to one aspect of the present invention, there is provided a protein or polypeptide selected from the group consisting of: (a) Placental Protein 13 (PP13) having the amino acid sequence shown in Fig. 2 (SEQ.ID.NO: 9); (b) a polypeptide having a sequence of amino acids included in PP13 and which binds to antibodies which specifically bind to PP13; (c) a protein or polypeptide of (a) or (b) in which one or more amino acids have been added, deleted or replaced without reducing the ability of the protein or polypeptide to bind antibodies which specifically bind to PP13; and (d) a protein or polypeptide having an amino acid sequence including the amino acid sequence of (a) or (b) or (c).

By another aspect of the present invention, there is provided a DNA molecule encoding the above protein or polypeptide.

According to another aspect of the present invention, there is provided a method of screening for pregnancy-related complications comprising the steps of: (a) providing a serum sample of a pregnant woman; (b) determining the level of PP13 or a peptide derived therefrom in the serum sample, and (c) comparing the determined level with pre-determined normal levels for women at the same

gestational age, a deviation between the levels being indicative of a pregnancy-related complication.

By one embodiment of the invention, the determination in step (b) is by means of antibodies, preferably monoclonal antibodies, directed against said proteins or polypeptides.

According to yet another aspect of the present invention, there is provided a recombinant method for the production of PP13 comprising inserting said DNA molecule into an expression vector, inserting the expression vector into a host cell, and incubating the host cell under conditions which permit expression of the inserted vector.

The present invention provides for the first time the full amino acid sequence of PP13, as well as its full cDNA sequence. This information can be utilized in a number of applications. For example, modified PP13 protein homologues and analogues can be produced in which one or more amino acids have been added, deleted or replaced, the modified protein typically retaining 75% homology with PP13. Methods for modifying the amino acid sequence of a protein whose full sequence is known are well known in the art, and include e.g. chemical synthesis, controlled mutagenesis and recombinant methods. Such modified proteins may have superior properties over the natural PP13 in various applications, such as superior immunogenicity or immuno-specificity (e.g. the modified protein may be devoid of immune epitopes common with other proteins) for use in an immunoassay for the early detection of pregnancy-related disorders as described in Silberman.

Furthermore, peptide fragments may be prepared from PP13 and such peptides may be modified as described above with respect to the full protein. These peptides may also be used in various applications. For example, it is well known that immunogenic proteins have specific amino acid sequences or epitopes which induce the immune system to mount an immune response to the protein. The above peptides may be tested for the presence of an epitope of PP13 so as to identify the epitope(s). A peptide containing an epitope may then be used in an immunoassay for pregnancy disorders. A number of PP13-derived peptides are disclosed below.

The pure PP13 protein or a derived peptide may be used to prepare antibodies to PP13. Either polyclonal or monoclonal antibodies may be produced by standard methods well known to the skilled artisan.

Both the antibodies as well as the proteins and peptides may be used to prepare diagnostic or screening assays for the detection of pregnancy-related complications such as intrauterine growth retardation, preterm delivery and preeclampsia. Examples of such assays are detailed in Silberman, the contents of which are incorporated herein by reference, and include radioimmunoassays (RIA) and enzyme-linked immunoassays (ELISA). In general, such an assay will include the steps of obtaining a serum sample of a pregnant woman, determining the level of PP13 or of a derived peptide in the serum sample by the immunoassay, and comparing the determined level with pre-determined normal levels for women at the same gestational age. A statistically significant deviation between the levels will be indicative of a pregnancy-related complication.

As mentioned above, the full cDNA of PP13 is disclosed here for the first time. Since the full amino acid sequence of PP13 is also disclosed, various DNA molecules encoding PP13 may be prepared due to the degeneracy of the genetic code. In addition, DNA molecules capable of hybridizing to these DNA molecules under stringent conditions may also be prepared. The DNA molecules may be used in a recombinant method for the production of PP13. Such methods are well known in the art and usually involve inserting the DNA molecule into an expression vector such as a plasmid, phage or viral DNA. The expression vector is then inserted into a compatible host cell such as bacterial cells, or eukaryotic cells such as yeast, plant, mammalian or insect cells. The host cell is incubated under conditions which induce expression of the inserted vector, thereby producing PP13.

For example, the DNA encoding PP13 can be inserted into an expression vector under the control of an inducible promotor such as the LacZ promoter, T7 or T4 polymerase promoter, heatshock promoters, etc. One example of an expression vector is the pQE expression vector (QIAGEN). The pQE vector provides high level expression of proteins containing a 6*His affinity tag in *E. coli*. The pQE contains a regulatable promoter consisting of the *E. coli* phage T5

promoter and two *lac* operator sequences. The vector is then inserted into a competent M15 [PREP4] *E. coli* strain (Villarejo and Zabin, 1974). The M15 host cell contains multiple copies of the plasmid pREPA which carries the *lacI* gene encoding the lac repressor. The host cell is incubated with IPTG which rapidly induces expression of the inserted vector, thereby producing PP13. Many other systems may also be used for PP13 expression, as is well known to the skilled artisan.

A kit for diagnosing pregnancy-related complications may be produced based on the present invention. Such a kit, for example, may comprise the following components: (1) antibodies capable of specifically binding PP-13; (2) labeled PP-13, for example by a radioactive, fluorescent or enzyme marker; (3) PP-13 standard solutions at known concentrations; and (4) means for detecting the signal produced in the assay. Such means could be, for example, antiserum raised against the PP-13-binding antibodies.

DETAILED DESCRIPTION OF THE DRAWINGS

The present invention will be better understood from the following detailed description of preferred embodiments, taken in conjunction with the following drawings in which:

Fig. 1 shows a partial nucleotide and deduced amino acid sequence of a cDNA from the Expressed Sequence Tag (EST) database (accession R24614). Regions that are similar to the sequenced peptides are underlined. PP13 derived peptide #3 (Fig 1) was found to share partial identity with this cDNA (red underlined letters), and peptides #4, #5 and #6 are 100% identical to the EST database sequence. The nucleotide sequence of the 390-bp cDNA is shown with a translation of the open reading frame (118 amino acids). A Kozak-like translation initiation sequence containing a presumptive start codon (ATG) at nucleotide 33 is labeled with an asterisk. Nucleotide numbers are shown on the left.

Fig. 2 shows the complete nucleotide and deduced amino acid sequence of the PP13 cDNA clone as obtained from RACE analysis. The nucleotide sequence of the 611-bp cDNA is shown with translation of the open reading frame (139 amino

acids). Regions that are identical to the digested peptide are numbered and underlined. A Kozak-like initiation of translation sequence containing a presumptive start codon (ATG) at nucleotide 41 is signed with asterisk. Nucleotide numbers are shown on the left.

Fig. 3 shows the alignment of amino acid sequence of PP13 and eosinophil lysophospholipase (SEQ. ID. NO: 11). Identical amino acids of PP13 protein and eosinophil lysophospholipase (EPL) are designated by bold. There is about 54% identity between the two proteins.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

MATERIALS AND METHODS

Materials

Modified trypsin and LysC (sequencing grade) were from Promega. Trifluoroacetic Acid (TFA) and hydrogenated Triton*X100 (RTX) were from Sigma. Ammonium Carbonate (AC) was from Riedel-de Haen. Acetonitrile (ACN) was from BioLab. 5' and 3' RACE Systems were from Gibco BRL. pUC57 cloning vector (T-Cloning Kit) was from MBI Fermentas.

Sequencing the PP13 protein

The PP13 protein was immuno-affinity purified using rabbit polyclonal antibodies raised against placental proteins and affinity purified on the PP-13 protein. In order to further purify the PP-13 protein and to digest it with proteolytic enzymes, we used the method of Rosenfeld et al. (1992) as follows. The PP-13 protein was separated from other contaminating proteins by resolving it on SDS-PAGE in a mini gel format (10x10cm) followed by fixing the gel and staining the gel with Coomassie brilliant blue. The gel was destained in 40% ethanol + 10% acetic acid. The stained gel band containing the PP-13 protein was cut out with a clean razor blade and washed with 50% acetonitrile (ACN) + 200mM Ammonium Carbonate (AC) in water. This treatment was performed in order to remove as much as possible of the SDS, Coomassie brilliant blue and acetic acid. The washed gel piece was air dried for 30 minutes and rehydrated by adding to it 50-100 μl of 200 mM AC + 1% RTX buffer containing 0.5 μgr modified trypsin or 0.5 μgr of LysC.

*Trade-mark

After incubation with gentle shaking at 37°C for 12 hours the proteolytic peptides released from the PP-13 protein were eluted from the gel piece by shaking it twice in 100 µl of 0.1% TFA + 60% ACN at room temperature for 60 min. The solution was separated from the gel piece by centrifugation and dried down in a Speed-Vac to remove excess ACN. The proteolytic peptides were resolved by Reverse-phase HPLC on a Vydac 1x150 mm, C18, 300 Å column with a linear gradient from 4% ACN + 0.1% TFA to 60% ACN + 0.085% TFA at room temperature with a flow rate of 40 µl/min. The elution pattern of the peptides was determined by UV absorbance at 214 nm and fractions containing peptides were collected by hand into microfuge tube and stored at -80°C. Some of the fractions containing peptides were sequenced on a Protein-Peptide Sequencer (models 476A and 494A, Perkin Elmer) using the manufacturer's standard Edman chemistry and cycles.

cDNA 3' and 5' ends analysis

In order to isolate the full cDNA sequence of the PP-13 gene, we used a standard method called Rapid Amplification of cDNA Ends (RACE) (2) to extend both the 5' and 3' ends of the known parts of the cDNA to its ends. Generally, the RACE method generates cDNA by using a Polymerase Chain Reaction (PCR) to amplify copies of the region between known segments of the cDNA at specific points in the transcript and its 3' or 5' ends. This was accomplished by making copies of the cDNA between synthetic DNA primers complementary to known segments of the message to primers that anneal to the ends of the cDNA.

For the 3' prime end determination, reverse transcriptase (RT) reaction was carried out using 4 µgr of total placental RNA (prepared by TRI reagent from Molecular Research Center, Inc.) and the 3' end primer: (106ras) 5'- ggc cac gcg tcg act agt act ttt ttt ttt ttt tt - 3'. This was followed by a PCR reaction between the primers: (107ras for the forward reaction) 5'- ggc cac gcg tcg act agt ac - 3' and the reverse primer (100rs, homologous to peptide # 4) was 5'- ggg ata tgg atg ttg gag gag ac - 3'. The PCR reaction included 2.5 mM $MgCl_2$, denaturation at 94°C for 45", primer annealing at 60°C for 45" and primer extension at 72°C for 2 min. for 35 cycles.

For the 5' end determination the RT reaction was carried out with 4 µgr

of total placental RNA and a specific 3' primer (101ras): 5'- gtc tcc tcc aac atc cat atc - 3'. The 5' end of the cDNA was extended by adding to it poly-dC using the RACE protocol and reagents (Gibco BRL). This was followed by a PCR reaction using conditions as above and the following primers: a backward primer with the abridged anchor primer (AAP) supplied by Gibco BRL and the forward reaction primer 101rs described above.

The resulting PCR fragments were inserted into the pUC57-T cloning vector (T-Cloning Kit #K1212 MBI Fermentas) and clones containing the insert were selected and sequenced by automated DNA at the Biological Services at the Weizmann Institute, Rehovot, Israel.

RESULTS

Identification of peptides from PP13 Protein

In order to either clone the gene encoding the PP-13 protein or to identify its gene in one of the data banks, it was necessary to obtain the primary amino acid sequence of the PP-13 protein. Since the PP13 protein was blocked at its amino terminus, internal amino acid sequences were obtained after proteolytically digesting the protein into peptide fragments. These peptides were separated and purified by chromatography using reverse-phase HPLC, and some of the resolved peptides were sequenced. The amino acid sequences of the peptides that were successfully sequenced are listed in Table 1.

Table 1. Amino acid sequences of PP13 derived peptide fragments obtained after trypsin and LysC digestion as described above.

Peptide number	Amino acid sequence
1. (SEQ.ID.NO: 1)	**L P V S L S V G**
2. (SEQ.ID.NO: 2)	**V I I K**
3. (SEQ.ID.NO: 3)	**G T P I H S F I N D P Q L Q V D F**
4. (SEQ.ID.NO: 4)	**E F G I W M L E E T T D Y V P F E**
5. (SEQ.ID.NO: 5)	**Q F E L C I Y**
6. (SEQ.ID.NO: 6)	**V H Y N E Y**
7. (SEQ.ID.NO: 7)	**G F V H R**

Comparing peptides sequence to Data-Banks

DNA and protein data banks available through the Internet were searched for homology to the obtained PP-13 peptides sequences. A cDNA sequence (SEQ.ID.NO: 8) encoding four of the peptides fragments (Fig 2) was identified (EST accession R24614). The fact that homology to more than one peptide sequence was present in the identified cDNA indicates that this cDNA is likely a product of the gene encoding the protein which is the major constituent of the PP13 preparation.

The sequence was found in an EST data bank created by the University of Washington and searched through the National Center for Biotechnology Information (NCBI) using the BLAST search program. The R24614 cDNA contains a Kozak-like translation initiation sequence and a 358 base-pair open reading frame (ORF) encoding a 118 amino acid polypeptide. The calculated molecular weight of the polypeptide encoded by the R24614 open reading frame is 13.9 Kda. Four of the sequenced peptides have homology to parts of the deduced sequence of the large open reading frame of the R24614 cDNA (Fig 1). The obtained amino acid sequence of peptide #3 was found to share partial identity with the EST cDNA and peptides number 4, 5 and 6 were identical to different segments of the ORF in the R24614 sequence.

Since the open reading frame sequence of R24614 obtained from the data bank did not contain the entire coding region of the PP13 protein, it was necessary to obtain the full cDNA sequence.

Identification of PP13 complete cDNA sequence

In order to obtain full cDNA sequence we used Rapid Amplification of cDNA ends (RACE). Using the RACE method with an internal specific primers homologous to the sequence from the region of peptide 4 previously found (Fig 1), we discovered the 3' and 5' end of PP13 message. The full PP13 amino acid sequence (SEQ.ID.NO: 9) and cDNA (SEQ.ID.NO: 10) are shown in Figure 2.

The full cDNA contains a Kozak-like translation initiation sequence and a 417-bp open reading frame encoding a 139 amino acid polypeptide, with a predicted mass of 15.1 KDa which is about the same size of the molecular weight of the PP13 protein as calculated from its migration in SDS-PAGE. The major open reading frame of the full cDNA sequence contains all of the peptides sequence previously found by Edman sequencing of reverse-phase purified proteolytic peptides (Fig 1).

Resemblance to other proteins

It turned out that the novel gene contains sequence similarity to eosinophil lysophospholipase (3), a protein of known significance in immunity and pregnancy disorders (Fig 3). PP13 and eosinophil lysophospholipase have about 54% amino acid identity and 56% nucleic acid identity. The identity of the two proteins in the regions of the peptides, especially peptides number 4 and 6 is low, so it is clear that these proteins are different, but the homology and identity might suggests they belong to the same protein family.

References:

1. Rosenfeld et al. (1992) In-Gel digestion of protein for internal sequence analysis after one or two dimensional Gel Electrophoresis. Analytical Biochemistry, 203, 173-175.

2. Frohman, M.A., (1990) PCR Protocols: A Guide to Methods and Applications (Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J, eds.) p. 28, Academic Press, San Diego.

3. Ackerman, S.J., Corrette, S.E., Rosenberg, H.F., Bennett, J.C., Mastrianni, D.M., Nicholson-Weller, A., Weller, P.F., Chin, D.T., and Tener, D.G. (1993) The J. of Immunology, 150, No. 2, pp 456-468.

4. Villarego, M.R. and Zabin, I. (1974) J. Bacteriol., 120, 466-474.

SEQUENCE LISTING

<110> DIAGNOSTIC TECHNOLOGIES LTD.

<120> Placental protein 13

<130> Diagnostic Technologies Ltd.

<140>
<141>

<150> 123098
<151> 1998-01-29

<160> 11

<170> PatentIn Ver. 2.0

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1 5

<210> 2
<211> 4
<212> PRT
<213> Human placental tissue

<220>
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<400> 2
Val Ile Ile Lys
1

<210> 3
<211> 17
<212> PRT
<213> Human placental tissue

<220>
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<222> (1)..(17)
<223> PP-13 derived (Page 8)

<400> 3
Gly Thr Pro Ile His Ser Phe Ile Asn Asp Pro Gln Leu Gln Val Asp
1 5 10 15

Phe

<210> 4
<211> 17
<212> PRT
<213> Human placental tissue

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Glu

<210> 5
<211> 7
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1 5

<210> 6
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<400> 6
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1 5

<210> 7
<211> 5
<212> PRT
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<220>
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<400> 7
Gly Phe Val His Arg
1 5

<210> 8
<211> 611
<212> DNA
<213> Human placental tissue

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<221> gene
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<223> PP-13 clone R24614 (Fig. 2)

<400> 8
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atacaaactg cctgtgtctt tgtctgttgg ttcctgcgtg ataatcaaag ggacaccaat 120
ccactctttt atcaatgacc cacagctgca ggtggatttc tacactgaca tggatgagga 180
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tgagtttggg atatggatgt tggaggagac aacagactac gtgccctttg aggatggcaa 300
acaatttgag ctgtgcatct acgtacatta caatgagtat gagataaagg tcaatggcat 360
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aaaaaaaaaa a 611

<210> 9
<211> 139
<212> PRT
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<220>
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<223> PP-13 (Fig.2)

<400> 9

Met	Ser	Ser	Leu	Pro	Val	Pro	Tyr	Lys	Leu	Pro	Val	Ser	Leu	Ser	Val
1				5				10						15	

Gly	Ser	Cys	Val	Ile	Ile	Lys	Gly	Thr	Pro	Ile	His	Ser	Phe	Ile	Asn
			20					25					30		

Asp	Pro	Gln	Leu	Gln	Val	Asp	Phe	Tyr	Thr	Asp	Met	Asp	Glu	Asp	Ser
		35					40					45			

Asp	Ile	Ala	Phe	Arg	Phe	Arg	Val	His	Phe	Gly	Asn	His	Val	Val	Met
	50					55					60				

Asn	Arg	Arg	Glu	Phe	Gly	Ile	Trp	Met	Leu	Glu	Glu	Thr	Thr	Asp	Tyr
65					70					75					80

Val	Pro	Phe	Glu	Asp	Gly	Lys	Gln	Phe	Glu	Leu	Cys	Ile	Tyr	Val	His
				85					90					95	

Tyr	Asn	Glu	Tyr	Glu	Ile	Lys	Val	Asn	Gly	Ile	Arg	Ile	Tyr	Gly	Phe
			100					105					110		

Val	His	Arg	Ile	Pro	Pro	Ser	Phe	Val	Lys	Met	Val	Gln	Val	Ser	Arg
		115					120					125			

Asp	Ile	Ser	Leu	Thr	Ser	Val	Cys	Val	Cys	Asn
	130					135				

<210> 10

<211> 417
<212> DNA
<213> Human placental tissue

<220>
<221> gene
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<223> PP-13 (Fig. 2)

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ataatcaaag ggacaccaat ccactctttt atcaatgacc cacagctgca ggtggatttc 120
tacactgaca tggatgagga ttcagatatt gccttccgtt tccgagtgca ctttggcaat 180
catgtggtca tgaacaggcg tgagtttggg atatggatgt tggaggagac aacagactac 240
gtgccctttg aggatggcaa acaatttgag ctgtgcatct acgtacatta caatgagtat 300
gagataaagg tcaatggcat acgcatttac ggctttgtcc atcgaatccc gccatcattt 360
gtgaagatgg tgcaagtgtc gagagatatc tccctgacct cagtgtgtgt ctgcaat 417

<210> 11
<211> 142
<212> PRT
<213> Human white blood cells

<220>
<221> PEPTIDE
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<223> Eosinophil Lysophospholipase (Fig. 3)

<400> 11
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1 5 10 15

Gly Ser Thr Val Thr Ile Lys Gly Arg Pro Leu Val Cys Phe Leu Asn
20 25 30

Glu Pro Tyr Leu Gln Val Asp Phe His Thr Glu Met Lys Glu Glu Ser
35 40 45

Asp Ile Val Phe His Phe Gln Val Cys Phe Gly Arg Arg Val Val Met
50 55 60

Asn Ser Arg Glu Tyr Gly Ala Trp Lys Gln Gln Val Glu Ser Lys Asn
65 70 75 80

Met Pro Phe Gln Asp Gly Gln Glu Phe Glu Leu Ser Ile Ser Val Leu
85 90 95

Pro Asp Lys Tyr Gln Val Met Val Asn Gly Gln Ser Ser Tyr Thr Phe

```
                100                 105                 110

Asp His Arg Ile Lys Pro Glu Ala Val Lys Met Val Gln Val Trp Arg
        115                 120                 125

Asp Ile Ser Leu Thr Lys Phe Asn Val Ser Tyr Leu Lys Arg
    130                 135                 140
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CLAIMS:

1. A recombinant protein or polypeptide selected from:

(a) Placental Protein 13 (PP 13) of the amino acid sequence as set forth in SEQ ID NO:9; and

(b) a protein or polypeptide having an amino acid sequence including the amino acid sequence of (a).

2. A DNA molecule encoding the protein or polypeptide of claim 1.

3. The DNA molecule according to claim 2 having the nucleotide sequence as set forth in SEQ ID NO:10.

4. A recombinant method for the production of Placental Protein 13 (PP 13) comprising:

(a) inserting a DNA molecule according to claim 2 into an expression vector;

(b) inserting said expression vector into a host cell; and

(c) incubating said host cell to express the inserted vector, thereby producing PP 13.

5. A kit for diagnosing pregnancy-related complications comprising:

(a) an antibody that specifically binds the protein or polypeptide of claim 1;

(b) labelled Placental Protein 13 (PP 13) as defined in claim 1; and

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(c) standard solution of recombinant PP 13 at known concentrations.

SMART & BIGGAR

OTTAWA, CANADA

PATENT AGENTS

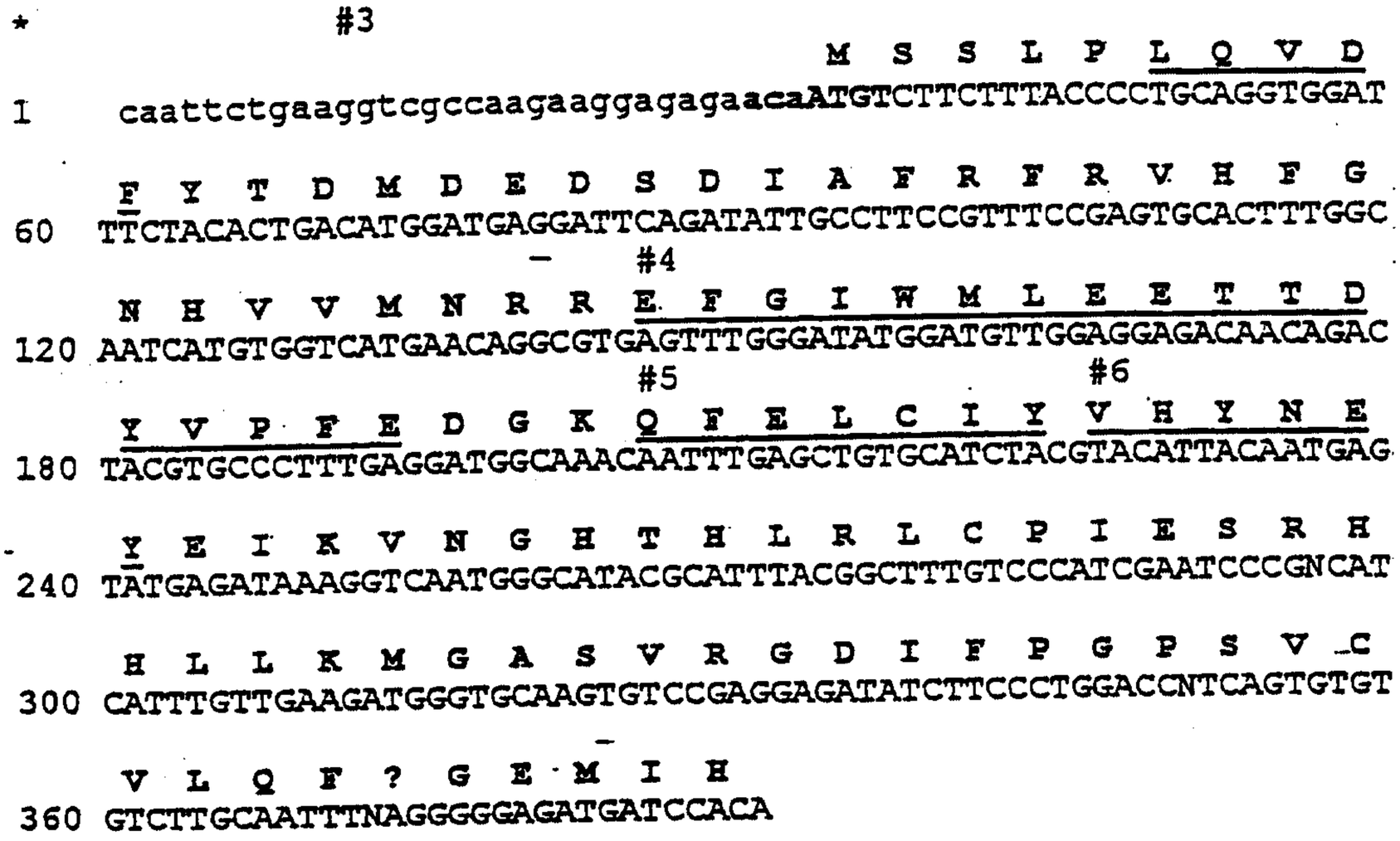
* #3
M S S L P L Q V D
1 caattctgaaggtcgccaagaaggagagaacaATGTCTTCTTTACCCCTGCAGGTGGAT
F Y T D M D E D S D I A F R F R V H F G
60 TTCTACACTGACATGGATGAGGATTCAGATATTGCCTTCCGTTTCCGAGTGCACTTTGGC
#4
N H V V M N R R E F G I W M L E E T T D
120 AATCATGTGGTCATGAACAGGCGTGAGTTTGGGATATGGATGTTGGAGGAGACAACAGAC
#5 #6
Y V P F E D G K Q F E L C I Y V H Y N E
180 TACGTGCCCTTTGAGGATGGCAAACAATTTGAGCTGTGCATCTACGTACATTACAATGAG
Y E I K V N G H T H L R L C P I E S R H
240 TATGAGATAAAGGTCAATGGGCATACGCATTTACGGCTTTGTCCCATCGAATCCCGNCAT
H L L K M G A S V R G D I F P G P S V C
300 CATTTGTTGAAGATGGGTGCAAGTGTCCGAGGAGATATCTTCCCTGGACCNTCAGTGTGT
V L Q F ? G E M I H
360 GTCTTGCAATTTNAGGGGGAGATGATCCACA

FIG. 1

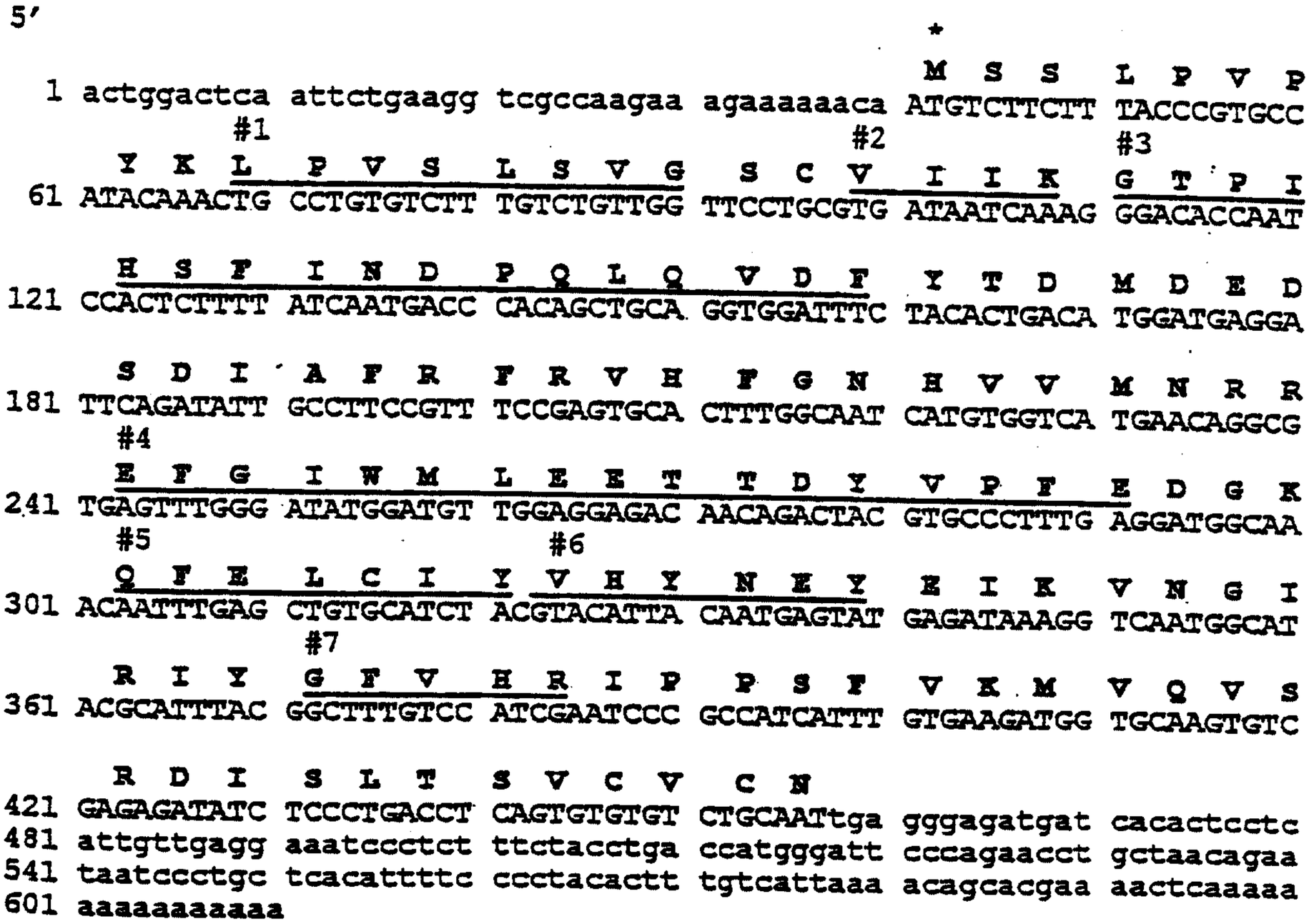
5′
M S S L P V P
1 actggactca attctgaagg tcgccaagaa agaaaaaaca ATGTCTTCTT TACCCGTGCC
#1 #2 #3
Y K L P V S L S V G S C V I I K G T P I
61 ATACAAACTG CCTGTGTCTT TGTCTGTTGG TTCCTGCGTG ATAATCAAAG GGACACCAAT
H S F I N D P Q L Q V D F Y T D M D E D
121 CCACTCTTTT ATCAATGACC CACAGCTGCA GGTGGATTTC TACACTGACA TGGATGAGGA
S D I A F R F R V H F G N H V V M N R R
181 TTCAGATATT GCCTTCCGTT TCCGAGTGCA CTTTGGCAAT CATGTGGTCA TGAACAGGCG
#4
E F G I W M L E E T T D Y V P F E D G K
241 TGAGTTTGGG ATATGGATGT TGGAGGAGAC AACAGACTAC GTGCCCTTTG AGGATGGCAA
#5 #6
Q F E L C I Y V H Y N E Y E I K V N G I
301 ACAATTTGAG CTGTGCATCT ACGTACATTA CAATGAGTAT GAGATAAAGG TCAATGGCAT
#7
R I Y G F V H R I P P S F V K M V Q V S
361 ACGCATTTAC GGCTTTGTCC ATCGAATCCC GCCATCATTT GTGAAGATGG TGCAAGTGTC
R D I S L T S V C V C N
421 GAGAGATATC TCCCTGACCT CAGTGTGTGT CTGCAATtga gggagatgat cacactcctc
481 attgttgagg aaatccctct ttctacctga ccatgggatt cccagaacct gctaacagaa
541 taatccctgc tcacattttc ccctacactt tgtcattaaa acagcacgaa aactcaaaaa
601 aaaaaaaaaaa

FIG. 2

```
PP13   1 MSSLPVPYKLPVSLSVGSCVIIKGTPIHSFINDPQLQVDFYTDMDED
         || | ||||     ||| || | ||| |    | | | |||||  | | |
EPL    1 MSLLPVPYTEAASLSTGSTVTIKGRPLVCFLNEPYLQVDFHTEMKEE

PP13  48 SDIAFRFRVHFGNHVVMNRREFGIWMLEETTDYVPFEDGKQFELCIY
         ||| |  | | ||   |||| || | |           || +|  ||| |
EPL   48 SDIVFHFQVCFGRRVVMNSREYGAWKQQVESKNMPFQDGQEFELSIS

PP13  95 VHYNEYEIKVNGIRIYGFVHRIPPSFVKMVQVSRDISLTSVCVCN
         |    |   |||   |   ||| | ||| ||| || +|||   |
EPL   95 VLPDKYQVMVNGQSSYTFDHRIKPEAVKMVQVWRDISLTKFNVSYLKR
```

FIG. 3