(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 May 2007 (10.05.2007)

PCT

(10) International Publication Number
WO 2007/053161 A2

(51) International Patent Classification:
G01N 33/53 (2006.01)

(21) International Application Number:
PCT/US2005/045805

(22) International Filing Date:
15 December 2005 (15.12.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/636,275 15 December 2004 (15.12.2004) US

(71) Applicant *(for all designated States except US)*: **BETH ISRAEL DEACONESS MEDICAL CENTER** [US/US]; 330 Brookline Avenue, Boston, MA 02215 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KARUMANCHI, S., Ananth** [US/US]; 117 Woodcliff Road, Chestnut Hill, CA 02467 (US). **SUKHATME, Vikas, P.** [US/US]; 36 Sky View Circle, Newton, MA 02459 (US).

(74) Agent: **BIEKER-BRADY, Kristina**; Clark & Elbing LLP, 101 Federal Street, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/053161 A2

(54) Title: NUCLEIC ACIDS AND POLYPEPTIDES USEFUL FOR DIAGNOSING AND TREATING COMPLICATIONS OF PREGNANCY

(57) Abstract: Disclosed herein are methods for diagnosing or treating pregnancy related hypertensive disorders that include the use of a polypeptide or a nucleic acid encoding a polypeptide selected from the following: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1- anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehyrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11.

NUCLEIC ACIDS AND POLYPEPTIDES USEFUL FOR DIAGNOSING AND TREATING COMPLICATIONS OF PREGNANCY

Statement as to Federally Sponsored Research

This research was funded, in part, by NIH Grant R03 DK064255-02. The U.S. government has certain rights to the invention.

Field of the Invention

In general, this invention relates to the detection and treatment of subjects having a pregnancy related hypertensive disorder.

Background of the Invention

Pre-eclampsia is a syndrome of hypertension, edema, and proteinuria that affects 5 to 10% of pregnancies and results in substantial maternal and fetal morbidity and mortality. Pre-eclampsia accounts for at least 200,000 maternal deaths worldwide per year. The symptoms of pre-eclampsia typically appear after the 20th week of pregnancy and are usually detected by routine measuring of the woman's blood pressure and urine. However, these monitoring methods are ineffective for diagnosis of the syndrome at an early stage, which could reduce the risk to the subject or developing fetus, if an effective treatment were available.

Currently there are no known cures for pre-eclampsia. Pre-eclampsia can vary in severity from mild to life-threatening. A mild form of pre-eclampsia can be treated with bed rest and frequent monitoring. For moderate to severe cases, hospitalization is recommended and blood pressure medication or anticonvulsant medications to prevent seizures are prescribed. If the condition becomes life threatening to the mother or the baby the pregnancy is terminated and the baby is delivered pre-term.

The proper development of the fetus and the placenta is mediated by several growth factors or angiogenic factors. Careful regulation of angiogenic and mitogenic signaling pathways is critical for maintaining appropriate proliferation, migration, and angiogenesis by trophoblast cells in the developing placenta. While several of these factors, such as VEGF and PlGF, have been identified, there are still many proteins for which a role in the pathogenesis of pre-eclampsia or eclampsia has not yet been identified.

There is a need for methods of accurately diagnosing subjects at risk for or having pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, particularly before the onset of the most severe symptoms. A treatment that would save maternal and fetal lives and prevent premature deliveries is also needed.

Summary of the Invention

We have discovered a means for diagnosing and effectively treating pregnancy related hypertensive disorders, including pre-eclampsia and eclampsia. In some cases both the diagnosis and treatment may occur prior to the development of symptoms. Such early diagnosis and treatment could save maternal and fetal lives and prevent premature deliveries.

We have discovered that the levels of expression of genes encoding the following secreted gene products (with GenBank numbers shown in parentheses) were significantly upregulated in the placental samples taken from women with pre-eclampsia as compared to placental specimens obtained from normal pregnant patients: follistatin related protein (U76702), interleukin 8 (M28130), inhibin A (M13981), VEGF-C (U43142), angiogenin (M11567), beta fertilin (U38805), hypothetical protein (AL039458), leukocyte associated Ig-like receptor secreted protein (AF013250), erythroid differentiation protein (J03634), adipogenesis inhibitory factor (X58377), corticotropin releasing factor binding protein (X58022), alpha-1 anti-chymotrypsin (X68733), insulin-like growth factor binding protein-5 (L27559), CD33L (D86368), cytokine

receptor like factor 1 (AF059293), platelet derived endothelial growth factor (NP_001953), lysyl hydroxylase isoform 2 (U84573), stanniocalcin precursor (U25997), secreted frizzled related protein (AF056087), and galectin-3 (NM_002306). We have also discovered that expression levels of the gene for the following secreted gene products were significantly decreased in placental samples taken from women with pre-eclampsia: alpha defensin (L12691), ADAM-TS3 (AB002364), cholecystokinin precursor (AW043690), interferon stimulated T-cell alpha chemoattractant precursor (AF030514), and azurocidin (M96326). These genes and the polypeptides encoded by the genes can be used to diagnose, treat, manage, and prevent pregnancy related hypertensive disorders.

We have also discovered intracellular targets that are differentially expressed in pre-eclamptic placentas and are suitable candidates for screening of novel therapeutic compounds. The intracellular gene products that are increased in pre-eclamptic placentas are: sperminine oxidase (U01134), UDP glycosyltransferase 2 family polypeptide B28 (AF 091582), neurotrophic tyrosine kinase receptor 2 (X 63759), neutral endopeptidase (J03779), CDC28 protein kinase regulatory subunit 2 (X54942) and beta glucosidase (J03060). The intracellular gene products that are decreased in pre-eclamptic placentas are: lanosterol synthase (U22526), calcium/calmodulin-dependent serine protein kinase (AI688589), estrogen receptor-alternatively spliced transcript H (X86816), chemokine (CX3C motif) receptor 1 (U27699), tyrosinase-related protein 1 (M20681), hydoxy-delta-5-steroid dehydrogenase (AL080151), dihydropyramidinase-like-4 (J03634), and cytochrome P450-family 11 (D84361).

For the purposes of the descriptions below, all of the polypeptides described above are collectively referred to as "the polypeptides of the invention." The polypeptides are further grouped as "secreted polypeptides" and "intracellular polypeptides" as described above. While the detailed description presented herein refers specifically to polypeptides associated with

specific GenBank accession numbers, it will be clear to one skilled in the art that the detailed description can also apply to family members, isoforms, homologs, and/or variants that are substantially identical to the specified polypeptides.

Based on this data, we have discovered that compounds that decrease the levels or biological activity of a polypeptide of the invention for which the gene was upregulated in pre-eclampsia can be used to treat or prevent pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, in a subject. Similarly, we have discovered that compounds that increase the levels or biological activity of a polypeptide of the invention for which the gene was downregulated in samples from women with pre-eclampsia can be used to treat or prevent pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, in a subject. Such agents include, but are not limited to, antibodies specific to the protein, nucleobase oligomers for antisense or RNAi targeting the protein, purified proteins, purified natural or synthetic compounds, chemical compounds, and small molecules.

Accordingly, the invention features methods for measuring the levels of any one or more of the polypeptides (secreted or intracellular) of the invention or a nucleic acid encoding a polypeptide of the invention as a detection tool for early diagnosis and management of pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia.

In one aspect, the invention features a method of diagnosing a subject as having or having a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes measuring the level of any one or more of the following secreted or intracellular polypeptides, or fragments thereof, in a sample from the subject: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (GenBank Accession Number AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-

chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta-glucosidase. In this method, an increase (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) in the level of any one or more of the above polypeptides, or fragments thereof, as compared to a normal reference sample, standard or level is a diagnostic indicator of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. The method can also include measuring two, three, four, or five or more of the secreted or intracellular polypeptides listed above, or fragments thereof. In preferred embodiments, the polypeptide is follistatin related protein, inhibin-A, beta fertilin, insulin-like growth factor binding protein-5, or secreted frizzled related protein.

Non-limiting examples of pregnancy related hypertensive disorders include pre-eclampsia, eclampsia, gestational hypertension, chronic hypertension, HELLP syndrome, and pregnancy with a small for gestational age infant (SGA).

In a related aspect, the invention features a method of diagnosing a subject as having or having a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes measuring the level of any one ore more of the following secreted or intracellular polypeptides, or fragments thereof, in a sample from the subject: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related

protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. In this method, a decrease (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) in the level of any one or more of the above polypeptides, or fragments thereof, as compared to a normal reference sample, standard, or level is a diagnostic indicator of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

For any of the diagnostic methods that include measuring the level of a polypeptide or fragment thereof, the measuring can be done using an immunological assay (e.g., an ELISA or a western blot). The method can also include measuring two, three, four, or five or more of the secreted or intracellular polypeptides or the nucleic acids encoding the polypeptides listed above, or fragments thereof. The measuring can also be performed for more than one polypeptide at a time, using for example, microarrays which can be formatted as an array of binding molecules (e.g., an array of antibodies, also known as antibody arrays) to detect the polypeptides of the invention, or as an array of polypeptides of the invention, also known as protein arrays, which can be used to detect levels of antibodies to the polypeptides in a biological sample.

In another aspect, the invention features a method of diagnosing a subject as having or having a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes measuring the level of a nucleic acid molecule encoding any one of the following secreted or intracellular polypeptides, or fragments thereof, in a sample from the subject: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (GenBank Accession Number AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin

precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta-glucosidase. In this method, an increase (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) in the level of any one or more of the nucleic acid molecules encoding the above polypeptides, or fragments thereof, as compared to a normal reference sample, standard, or level is a diagnostic indicator of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In preferred embodiments, the nucleic acid encodes follistatin related protein, inhibin-A, beta fertilin, insulin-like growth factor binding protein-5, or secreted frizzled related protein.

In a related aspect, the invention features a method of diagnosing a subject as having or having a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes measuring the level of a nucleic acid molecule encoding any one of the following secreted or intracellular polypeptides, or fragments thereof, in a sample from the subject: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. In this method, a decrease (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) in the level of a nucleic acid molecule encoding any one or more of the above polypeptides, or fragments thereof, as compared to a normal reference sample, standard, or level is a diagnostic indicator of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

The methods above can also include measuring two, three, four, or five or more of the nucleic acids encoding the secreted or intracellular polypeptides listed above, or fragments thereof.

The diagnosis of a pregnancy related hypertensive disorder or a predisposition to a pregnancy related hypertensive disorder can result from an alteration (e.g., an increase or decrease) in the relative level of a polypeptide of the invention as compared to a normal reference sample or from the detection of an absolute level of a polypeptide of the invention that is above or below a normal reference level. The diagnosis can also result from an alteration in the level of a polypeptide as compare to the level in a prior sample obtained from the same subject. In additional preferred embodiments, the reference standard or level is a level or number derived from such a sample. In additional preferred embodiments, the reference sample is obtained at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 9 weeks, 12 weeks, 15 weeks, 18 weeks or more prior to the measuring of the levels for diagnosis. The reference standard or level can also be a value derived from a normal subject that is matched to the sample subject by at least one of the following criteria: gestational age of the fetus, age of the mother, blood pressure prior to pregnancy, blood pressure during pregnancy, BMI of the mother, weight of the fetus, prior diagnosis of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, and a family history of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In additional preferred embodiments, the reference sample is a sample taken from a non-pregnant subject; a pregnant subject that does not have a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia; or a purified protein at known normal concentrations or a level representative of any of the reference samples described above.

In additional preferred embodiments, the method further includes measuring the level of at least one of sFlt-1, VEGF, PlGF, or soluble endoglin

polypeptide in a sample from a subject as described in U.S. Patent Application Publication Numbers 20040126828, 20050025762, and 20050170444; PCT Publication Numbers WO 2004/008946 and WO 2005/077007; and U.S Patent Application Serial Number 11/235,577. The method can also include measuring the level of at least two of sFlt-1, VEGF, PlGF, or soluble endoglin polypeptide in a sample from a subject and calculating the relationship between the levels of sFlt-1, VEGF, PlGF, or soluble endoglin using a metric, where an alteration in the relationship between the levels in the subject sample relative to a reference sample diagnoses a pregnancy related hypertensive disorder or a predisposition to a pregnancy related hypertensive disorder. In preferred embodiments, the method also includes determining the body mass index (BMI), the gestational age (GA) of the fetus, or both and including the BMI or GA or both in the metric. For example, the metric can be a pre-eclampsia anti-angiogenic index (PAAI): [sFlt-1/VEGF + PlGF], a soluble endoglin anti-angiogenic index: (sFlt-1 + 0.25(soluble endoglin polypeptide))/PlGF, sFlt1/PLGF, (sFlt1+soluble endoglin)/PlGF, (sFlt1+soluble endoglin+follistatin related protein)/PlGF, or any combination thereof.

In another aspect, the invention provides a method of diagnosing a subject as having, or having a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes determining the nucleic acid sequence of a gene encoding a polypeptide selected from the group consisting of: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral

endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase. An alteration in the subject's nucleic acid sequence that is an alteration that increases the expression level or biological activity of the gene product in the subject diagnoses the subject with a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a propensity to develop such a condition.

In another related aspect, the invention features a method of diagnosing a subject as having, or having a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes determining the nucleic acid sequence of a gene encoding a polypeptide selected from the group consisting of: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. An alteration in the subject's nucleic acid sequence that is an alteration that decreases the expression level or biological activity of the gene product in the subject diagnoses the subject with a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

In preferred embodiments of any of the above aspects, the polypeptide or the nucleic acid encoding the polypeptide is follistatin related protein, inhibin-A, beta fertilin, insulin-like growth factor binding protein-5, or secreted frizzled related protein.

In additional embodiments of any of the above aspects, the levels are measured on two or more occasions and a change in the levels between measurements is a diagnostic indicator of pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such

as pre-eclampsia or eclampsia. In preferred embodiments, an alteration (e.g., an increase or a decrease of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) in the level of any of the polypeptides of the invention or nucleic acids encoding a polypeptide of the invention from the first measurement to the next measurement is a diagnostic indicator of pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. Desirably, the diagnostic methods are used to diagnose a pregnancy related hypertensive disorder prior to the onset of symptoms (e.g., at least 4, 5, 6, 7, 8, 9, or 10 weeks prior).

In various embodiments of any of the above diagnostic aspects, the pregnancy related hypertensive disorder is pre-eclampsia, eclampsia, gestational hypertension, chronic hypertension, HELLP syndrome, or pregnancy with an SGA infant.

In various embodiments of the above aspects, the sample is a bodily fluid (e.g., urine, blood, amniotic fluid, serum, saliva, plasma, or cerebrospinal fluid) of the subject in which the polypeptide or nucleic acid encoding a polypeptide of the invention is normally detectable. In additional embodiments, the sample is a tissue or a cell (e.g., placental tissue or placental cells, endothelial cells, leukocytes, and monocytes). In other embodiments of the above aspects, the subject is a pregnant human, a post-partum human, or a non-pregnant human. In other embodiments of the above aspects, the subject is a non-human (e.g., a cow, a horse, a sheep, a pig, a goat, a dog, or a cat). In one embodiment, the subject is a non-pregnant human and the method is used to diagnose a propensity to develop a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, prior to a pregnancy. In additional embodiments, the BMI or GA or both is also measured.

In another aspect, the invention provides a kit for the diagnosis of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject that includes at least one nucleic acid sequence, or a sequence complementary

thereto, that is selected from nucleic acids that encode the following group of polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450–family 11. The kit also includes directions for the use of the nucleic acid sequence, or sequence complementary thereto, for the diagnosis of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In preferred embodiments, the kit includes at least two, at least three, at least four, or at least five or more of the nucleic acid sequences.

In another aspect, the invention provides a kit for the diagnosis of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject comprising a component or reagent used to detect a polypeptide that is selected from the following group of polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein,

alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor–alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. The kit also includes directions for the use of the components to detect the polypeptide for the diagnosis of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In preferred embodiments, the kit includes components or reagents used to detect at least two, at least three, at least four, or at least five or more of the polypeptides of the invention. Preferred polypeptides or nucleic acids include follistatin related protein, inhibin-A, beta fertilin, insulin-like growth factor binding protein-5, or secreted frizzled related protein. In preferred embodiments, the components or reagents used to detect a polypeptide include a binding molecule, such as an antibody or antigen binding fragment that is specific for the polypeptide and the polypeptide is detected by any one of the following assays: an immunological assay, an enzymatic assay, or a colorimetric assay. The component or reagent can also be a polypeptide, or fragment thereof, that can bind to an antibody that specifically binds the polypeptide. Such a kit can be used to detect antibodies present in a bodily fluid sample from a subject that are indicative of levels of the protein in the subject.

In additional preferred embodiments of any of the above kit aspects of the invention, the kit also includes a reference sample, standard, or level. The reference sample, standard, or level can be a normal reference sample, standard

or level taken from a subject not having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a subject that is not pregnant. The reference sample can also be a purified polypeptide at a known normal concentration.

In preferred embodiments, the diagnostic kit is labeled or includes instructions for use in the diagnosis of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a predisposition to a pregnancy related hypertensive disorder, in a subject. In yet another embodiment, the diagnostic kit is labeled or includes instructions for use in therapeutic monitoring or therapeutic dosage determination. Desirably, the diagnostic kit includes a label or instructions for the use of the kit to determine the levels of a polypeptide of the invention of the subject sample and to compare those subject sample levels to a reference sample value or a standard curve of reference sample values, where the standard curve shows values indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, and normal values. It will be understood that the reference sample values will depend on the intended use of the kit. For example, in a kit used for diagnostic purposes, the subject sample can be compared to a reference value or reference sample for a polypeptide of the invention taken from a subject that does not have a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or is not pregnant. In another example, a kit used for therapeutic monitoring can have a reference value or reference sample that is a positive reference indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, wherein an alteration (increase or decrease) in the value of the subject sample relative to the reference sample can be used to indicate an improvement in the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or effective dosages of therapeutic compounds.

In a related aspect, the invention features a device for diagnosing a subject as having or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. The device includes a component useful for comparing the levels of a polypeptide of the invention or a nucleic acid encoding a polypeptide of the invention, wherein an alteration (increase or decrease) in the levels of a polypeptide of the invention is a diagnostic indicator of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in the subject. In preferred embodiments, the device includes a membrane in a lateral flow or dipstick format used to measure and compare polypeptide levels in urine sample. The device can also include components for comparing the levels of one or more polypeptides of the invention or nucleic acid molecules encoding the polypeptides of the invention and at least one of soluble endoglin sFlt-1, VEGF, and PlGF nucleic acid molecules or polypeptides in a sample from a subject, relative to a reference sample, wherein an alteration (increase or decrease) diagnoses a pregnancy related hypertensive disorder or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia in the subject. In a preferred embodiment the device includes a component or components for use with a metric to compare the levels of one or more polypeptides of the invention and at least one, and preferably two, of soluble endoglin, sFlt-1, VEGF, and PlGF polypeptides.

In another aspect, the invention features a nucleic acid array comprising one or more substrate supports which are stably associated with a plurality of polynucleotide probes, wherein the polynucleotide probes are capable of hybridizing under highly stringent conditions to RNA transcripts, or the complements thereof, of nucleic acids encoding any of the polypeptides of the invention.

In another aspect, the invention features a polypeptide array comprising one or more substrate supports which are stably associated with a plurality of polypeptides of the invention; variants of the polypeptides; antibodies specific for the polypeptides or variants; or any combination of the polypeptides, variants, or antibodies.

Each of the arrays described above can also include instructions for the use of the array for the diagnosis of a pregnancy related hypertensive disorder or a predisposition thereto.

Any of the diagnostic methods, kits, or arrays described herein can also be used to monitor a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject. In preferred embodiments, the diagnostic methods are used to monitor the subject during therapy or to determine effective therapeutic dosages. The level of a polypeptide of the invention or a nucleic acid encoding a polypeptide of the invention is measured alone or in combination with the levels of soluble endoglin, sFlt-1, VEGF, or PlGF protein or nucleic acids, or any combination thereof. In preferred embodiments the levels of are measured on two or more occasions and an alteration (increase or decrease) in the levels is a diagnostic indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In additional preferred embodiments, the levels are compared to a reference sample and an alteration (increase or decrease) in the levels of any of the polypeptides relative to the reference sample is a diagnostic indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In one embodiment, the level of at least one of the following polypeptides or nucleic acids encoding the following secreted or intracellular polypeptides, or fragments thereof, is measured during or after administering therapy and compared to the value before therapy: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing

factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta-glucosidase. In this embodiment, a decrease in the level of any one or more of the above polypeptides, or fragments thereof, as compared to the value before therapy indicates an improvement in the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

In another embodiment, the level of at least one of the following secreted or intracellular polypeptides or nucleic acid encoding the secreted polypeptides, or fragments thereof is measured during or after administering therapy and compared to the value before therapy: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor – alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450 –family 11. In this embodiment, an increase in the level of any one or more of the above polypeptides, or fragments thereof, as compared to the value before therapy indicates an improvement in the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

In preferred embodiments of the diagnostic monitoring methods of the invention that include the measurement of sFlt-1, VEGF, or PlGF, the method can include calculating the relationship between the levels of sFlt-1, VEGF, or PlGF using a metric, wherein an alteration in the relationship between said levels in the subject sample relative to a reference sample, is a diagnostic indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. One example of such a metric is the PAAI. In this example, a

decrease in the PAAI value of a subject (e.g., less than 20, preferably less than 10) indicates an improvement in the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. A decrease in the PAAI (e.g., less than 20, preferably less than 10) can also indicate an effective dosage of a therapeutic compound. In preferred embodiments of the aspects relating to diagnosis or monitoring of therapeutic treatments, polypeptides are measured using an immunological assay, such as ELISA or western blot, or a protein array or antibody array for the measurement of expression levels of more than one polypeptide. For any of the monitoring methods, the measuring of levels can be done on two or more occasions and a change in the levels between measurements is a diagnostic indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

In another aspect, the invention provides a method of treating or preventing a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject by administering to the subject a compound capable of decreasing the biological activity or the expression level of a polypeptide or nucleic acid molecule encoding a polypeptide selected from the group of secreted polypeptides consisting of: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3, where the administering is for a time and in an amount sufficient to treat or prevent a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject. In preferred embodiments, the compound is a nucleobase oligomer that is at least 90%, 95%, 96%, 97%, 98%, 99% or 100% complementary to at least a portion of the nucleic acid sequence

encoding any of the polypeptides listed above. The nucleobase oligomer can be an antisense nucleobase oligomer, preferably at least 90%, 95%, 96%, 97%, 98%, 99% or 100% complementary to at least 8 to 30 nucleotides of the desired nucleic acid sequence. The nucleobase oligomer can also be a double stranded RNA (dsRNA), preferably a small interfering RNA (siRNA) that is preferably at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 35, 45, or 50 nucleotides of the desired nucleic acid sequence.

In additional preferred embodiments of this aspect, the compound is an antibody or antigen-binding fragment, preferably a monoclonal antibody, that specifically binds any one of the following polypeptides, or fragments thereof: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin–3. In preferred embodiments, the antibody or antigen-binding fragment thereof is a human or humanized antibody.

In another aspect, the invention features a method of treating or preventing a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject by administering to the subject a compound capable of increasing the biological activity or the expression level of a polypeptide or nucleic acid molecule encoding a secreted polypeptide selected from the group consisting of: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin, where the administering is for a time and in an amount sufficient to treat or prevent a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in

a subject. In a preferred embodiment, the compound is a purified polypeptide selected from the group consisting of: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin. In various embodiments of any of the above aspects, the method further involves the step of administering to a subject an anti-hypertensive compound (e.g., adenosine, nifedipine, minoxidil, and magnesium sulfate). In other embodiments of the above aspects, the subject is a pregnant human, a post-partum human, a non-pregnant human, or a non-human (e.g., a cow, a horse, a sheep, a pig, a goat, a dog, or a cat). The therapeutic methods of the invention can be used to treat or prevent a pregnancy related hypertensive disorder that includes pre-eclampsia, eclampsia, gestational hypertension, chronic hypertension, HELLP syndrome, and pregnancy with an SGA infant. Preferred disorders are pre-eclampsia and eclampsia. In various embodiments of the above aspects, the method can be combined with the diagnostic methods of the invention, described below, to monitor the subject during therapy or to determine effective therapeutic dosages.

Any of the therapeutic aspects of the invention can also include administering one ore more additional compounds, such as a purified sFlt-1 antibody, a sFlt-1 antigen-binding fragment, nicotine, theophylline, adenosine, nifedipine, minoxidil, magnesium sulfate, vascular endothelial growth factor (VEGF), including all isoforms such as VEGF189, VEGF121, or VEGF165, or fragments thereof; placental growth factor (PlGF), including all isoforms and fragments thereof; a purified soluble endoglin antibody or soluble endoglin antigen-binding fragment; where the administering is for a time and in an amount sufficient to treat or prevent the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a subject. Preferred examples of such compounds are described in U.S. Patent Application Publication Numbers 20040126828, 20050025762, and 20050170444; PCT Publication

Numbers WO 2004/008946 and WO 2005/077007; and U.S Patent Application Serial Number 11/235,577. Desirably, the compound will be a compound capable of binding to sFlt-1 or decreasing sFlt-1 expression.

Any of the therapeutic aspects of the invention can be used alone or in combination with one or more additional methods (diagnostic or treatment) of the invention.

In another aspect, the invention provides a method of identifying a compound that ameliorates a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that includes contacting a cell that expresses a polypeptide of the invention or a nucleic acid molecule encoding a polypeptide of the invention with a candidate compound, and comparing the level of expression or biological activity of the polypeptide of the invention or the nucleic acid molecule encoding the polypeptide of the invention in the cell contacted by the candidate compound with the level of expression or biological activity in a control cell not contacted by the candidate compound, where an alteration in expression or biological activity of the polypeptide of the invention or the nucleic acid molecule encoding the polypeptide of the invention identifies the candidate compound as a compound that ameliorates the pregnancy related hypertensive disorder.

In one embodiment, the method is used to identify a compound that decreases the expression of a polypeptide, or fragment thereof, or a nucleic acid molecule encoding the polypeptide, or fragment thereof, selected from the following group of polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3, sperminine oxidase, UDP

glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta-glucosidase. In another embodiment, the method is used to identify a compound that promotes an increase in the expression of a polypeptide, or fragment thereof, or a nucleic acid molecule encoding the polypeptide, or fragment thereof, selected from the following group of polypeptides: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin, or the level of any one of the following intracellular polypeptides, or fragments thereof, in a sample from the subject: lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. The alteration can be, for example, in transcription, translation, protein stability, production, or biological activity.

For the purpose of the present invention, the following abbreviations and terms are defined below.

By "alteration" is meant a change (increase or decrease) in the expression levels of a gene or polypeptide as detected by standard art known methods such as those described below. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater change in expression levels. "Alteration" can also indicate a change (increase or decrease) in the biological activity of any of the polypeptides of the invention. Examples of biological activities include ligand binding, enzymatic activity, cell migration, cell proliferation, induction of endothelial dysfunction, or induction of an anti-angiogenic state. Biological activities can be measured, for example, by ligand binding assays; cell migration assays; assays for enzymatic activity (e.g., kinase activity); Scatchard plot analysis; immunoassays; cell proliferation assays such as BrdU labeling,

cell counting experiments, or quantitative assays for DNA synthesis such as ^{3}H thymidine incorporation; and angiogenesis assays that are standard in the art or are described herein. As used herein, an alteration includes a 10% change in biological activity, preferably a 25% change, more preferably a 40% change, and most preferably a 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater change in biological activity.

By "antisense nucleobase oligomer" is meant a nucleobase oligomer, regardless of length, that is complementary to the coding strand or mRNA of a nucleic acid encoding a polypeptide of the invention. The antisense nucleobase oligomer can also be targeted to the translational start and stop sites. Preferably the antisense nucleobase oligomer comprises from about 8 to 30 nucleotides. The antisense nucleobase oligomer can also contain at least 40, 60, 85, 120, or more consecutive nucleotides that are complementary to mRNA or DNA encoding the polypeptide of the invention, and may be as long as the full-length mRNA or gene.

By "body mass index" is meant a number, derived by using height and weight measurements, that gives a general indication of whether or not weight falls within a healthy range. The formula generally used to determine the body mass index is a person's weight in kilograms divided by a person's height in meters squared or weight (kg)/ (height (m))^{2}.

By "compound" is meant any small molecule chemical compound, antibody, nucleic acid molecule, polypeptide, or fragments thereof.

By "chimeric antibody" is meant a polypeptide comprising at least the antigen-binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

By "decrease" is meant the ability to cause an overall reduction, preferably of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of polypeptide or nucleic acid, detected

by the assays described herein (see "expression") or the biological activity of the polypeptide, detected by the assays described herein (see "biological activity"), as compared to a reference sample.

By "double-stranded RNA (dsRNA)" is meant a ribonucleic acid molecule comprised of both a sense and an anti-sense strand. dsRNAs can be used to mediate RNA interference.

By "expression" is meant the detection of a gene or polypeptide by standard art known methods. For example, polypeptide expression is often detected by immunoassays (e.g., ELISA or western blotting), DNA expression is often detected by Southern blotting or polymerase chain reaction (PCR), and RNA expression is often detected by northern blotting, PCR, or RNAse protection assays.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids or nucleotides up to the entire length of the polypeptide or nucleic acid molecule.

By "gestational age" is meant a reference to the age of the fetus, counting from the first day of the mother's last menstrual period usually referred to in weeks.

By "gestational hypertension" is meant the development of high blood pressure without proteinuria after 20 weeks of pregnancy.

By a "history of pre-eclampsia or eclampsia" is meant a previous diagnosis of pre-eclampsia or eclampsia or pregnancy induced hypertension in the subject themselves or in a related family member.

By "homologous" is meant any gene or polypeptide sequence that bears at least 30% homology, more preferably 40%, 50%, 60%, 70%, 80%, and most preferably 90% or more homology to a known gene or polypeptide sequence

over the length of the comparison sequence. A "homologous" polypeptide can also have at least one biological activity of the comparison polypeptide. For polypeptides, the length of comparison sequences will generally be at least 6 amino acids, preferably at least 10 or 20 amino acids, more preferably at least 25 amino acids, and most preferably 50, 100, 150, 200 amino acids or more, up to the entire length of the polypeptide. For nucleic acids, the length of comparison sequences will generally be at least 18 nucleotides, preferably at least 25 or 50 nucleotides, more preferably at least 75 nucleotides, and most preferably from at least 100, 150, 200, 250, 300 nucleotides or more up to the entire length of the nucleic acid. "Homology" can also refer to a substantial similarity between an epitope used to generate antibodies and the polypeptide or fragment thereof to which the antibodies are directed. In this case, homology refers to a similarity sufficient to elicit the production of antibodies that can specifically recognize the polypeptide at issue.

By "humanized antibody" is meant an immunoglobulin amino acid sequence variant or fragment thereof that is capable of binding to a predetermined antigen. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, or CH4 regions of the heavy chain. The humanized antibody comprises a framework region (FR) having substantially the amino acid sequence of a human immunoglobulin and a complementarity determining region (CDR) having substantially the amino acid sequence of a non-human immunoglobulin (the "import" sequences).

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally will comprise at least

a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. By "complementarity determining region (CDR)" is meant the three hypervariable sequences in the variable regions within each of the immunoglobulin light and heavy chains. By "framework region (FR)" is meant the sequences of amino acids located on either side of the three hypervariable sequences (CDR) of the immunoglobulin light and heavy chains.

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75%, preferably 90%, and most preferably at least 95%, 96%, 97%, 98%, 99% or 100% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences.

By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences, or portions thereof, under various conditions of stringency. (See, e.g., Wahl and Berger (1987) *Methods Enzymol.* 152:399; Kimmel, *Methods Enzymol.* 152:507, 1987.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of

stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci.*, USA 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York,

2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.

By "increase" is meant the ability to cause an overall increase preferably of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of polypeptide or nucleic acid, detected by the aforementioned assays (see "expression") or the biological activity of the polypeptide, detected by the aforementioned assays (see "biological activity"), as compared to a reference sample.

By "intrauterine growth retardation (IUGR)" is meant a syndrome resulting in a birth weight which is less that 10 percent of the predicted fetal weight for the gestational age of the fetus. The current World Health Organization criterion for low birth weight is a weight less than 2,500 gm (5 lbs. 8 oz.) or below the 10^{th} percentile for gestational age according to U.S. tables of birth weight for gestational age by race, parity, and infant sex (Zhang and Bowes, *Obstet. Gynecol.* 86:200-208, 1995). These low birth weight babies are also referred to as "small for gestational age (SGA)." Pre-eclampsia is a condition known to be associated with IUGR or SGA.

By "metric" is meant a measure. A metric may be used, for example, to compare the levels of a polypeptide or nucleic acid molecule of the invention. Exemplary metrics include, but are not limited to, mathematical formulas or algorithms, such as ratios. Depending on the metric that is used, the diagnostic indicator of eclampsia or pre-eclampsia may be significantly above or below a value using the same metric with a reference sample or level (e.g., from a control subject not having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia). The metric to be used is that which best discriminates between levels of a polypeptide or nucleic acid molecule of the invention, and/or soluble endoglin, sFlt-1, VEGF, PlGF, or any combination thereof, in a subject having pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, and a reference sample or level. For example, the

metric can be a pre-eclampsia anti-angiogenic index (PAAI): [sFlt-1/VEGF + PlGF], a soluble endoglin anti-angiogenic index: (sFlt-1 + 0.25(soluble endoglin polypeptide))/PlGF, sFlt1/PLGF, (sFlt1+soluble endoglin)/PlGF, (sFlt1+soluble endoglin+follistatin related protein)/PlGF, or any combination thereof. Some examples of metrics that are useful are described in U.S. Patent Application Publication Numbers 20040126828, 20050025762, and 20050170444; PCT Publication Numbers WO 2004/008946 and WO 2005/077007; and U.S Patent Application Serial Number 11/235,577.

By a "nucleobase oligomer" is meant a compound that includes a chain of at least eight nucleobases, preferably at least twelve, and most preferably at least sixteen bases, joined together by linkage groups. Included in this definition are natural and non-natural oligonucleotides, both modified and unmodified, as well as oligonucleotide mimetics such as Protein Nucleic Acids, locked nucleic acids, and arabinonucleic acids. Examples of numerous nucleobases and linkage groups that may be used in the nucleobase oligomers of the invention, can be found in U.S. Patent Application Publication Nos. 20030114412, paragraphs [0030] to [0046] and 20030114407, paragraphs [0036] to [0055], and 20030190659, paragraphs [0083] to [0106], herein incorporated by reference.

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

By "pharmaceutically acceptable carrier" is meant a carrier that is physiologically acceptable to the treated mammal while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier substance is physiological saline. Other physiologically acceptable carriers and their formulations are

known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (20th edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA.

By "polymorphism" is meant a genetic variation, mutation, deletion or addition in a nucleic acid molecule encoding a polypeptide of the invention that is indicative of a predisposition to develop a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. A polymorphism may be present in the promoter sequence, an open reading frame, intronic sequence, or untranslated 3' region of a gene.

By "pregnancy related hypertensive disorder" is meant any condition or disease or pregnancy that is associated with or characterized by an increase in blood pressure. Included among these conditions are pre-eclampsia (including premature pre-eclampsia, severe pre-eclampsia), eclampsia, gestational hypertension, HELLP syndrome, (hemolysis, elevated liver enzymes, low platelets), abruption placenta, chronic hypertension, pregnancy with intra uterine growth restriction, and pregnancy with a small for gestational age (SGA) infant. It should be noted that although pregnancy with a SGA infant is not often associated with hypertension, it is included in this definition.

By "pre-eclampsia" is meant the multi-system disorder that is characterized by hypertension with proteinuria or edema, or both, glomerular dysfunction, brain edema, liver edema, or coagulation abnormalities due to pregnancy or the influence of a recent pregnancy. Pre-eclampsia generally occurs after the 20th week of gestation. Pre-eclampsia is generally defined as some combination of the following symptoms: (1) a systolic blood pressure (BP) >140 mmHg and a diastolic BP >90 mmHg after 20 weeks gestation (generally measured on two occasions, 4-168 hours apart), (2) new onset proteinuria (1+ by dipstik on urinanaysis, >300mg of protein in a 24-hour urine collection, or a single random urine sample having a protein/creatinine ratio >0.3), and (3) resolution of hypertension and proteinuria by 12 weeks postpartum. Severe pre-eclampsia is generally defined as (1) a diastolic BP >

110 mmHg (generally measured on two occasions, 4-168 hours apart) or (2) proteinuria characterized by a measurement of 3.5 g or more protein in a 24-hour urine collection or two random urine specimens with at least 3+ protein by dipstick. In pre-eclampsia, hypertension and proteinuria generally occur within seven days of each other. In severe pre-eclampsia, severe hypertension, severe proteinuria and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) or eclampsia can occur simultaneously or only one symptom at a time. Occasionally, severe pre-eclampsia can lead to the development of seizures. This severe form of the syndrome is referred to as "eclampsia." Eclampsia can also include dysfunction or damage to several organs or tissues such as the liver (e.g., hepatocellular damage, periportal necrosis) and the central nervous system (e.g., cerebral edema and cerebral hemorrhage). The etiology of the seizures is thought to be secondary to the development of cerebral edema and focal spasm of small blood vessels in the kidney.

By "pre-eclampsia anti-angiogenesis index (PAAI)" is meant the ratio of sFlt-1/VEGF + PlGF used as an indicator of anti-angiogenic activity. A PAAI greater than 10, more preferably greater than 20, is considered to be indicative of pre-eclampsia or risk of pre-eclampsia.

By "premature pre-eclampsia" is meant pre-eclampsia with onset of symptoms <37 weeks or <34 weeks.

By "protein" or "polypeptide" or "polypeptide fragment" is meant any chain of more than two amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally occurring polypeptide or peptide, or constituting a non-naturally occurring polypeptide or peptide.

By "polypeptide of the invention" is meant any of the following secreted polypeptides where the number in parenthesis indicates the GenBank accession number for the polypeptide: follistatin related protein (FLRG, U76702), interleukin 8 (IL-8, M28130), inhibin A (M13981), VEGF-C (U43142), angiogenin (M11567), beta fertilin (U38805), hypothetical protein

(AL039458), leukocyte associated Ig-like receptor secreted protein (LAIR-2, AF013250), erythroid differentiation protein (J03634), adipogenesis inhibitory factor (X58377), corticotropin releasing factor binding protein (CRF-BP, X58022), alpha-1 anti-chymotrypsin (X68733), insulin-like growth factor binding protein-5 (IGFBP-5, L27559), CD33L (D86358), cytokine receptor like factor 1 (CRLF1, AF059293), platelet derived endothelial growth factor (ECGF-1, NP_001953), lysyl hydroxylase isoform 2 (PLOD2, U84573), stanniocalcin precursor (U25997), secreted frizzled related protein (AF056087), galectin -3 (NM_002306), alpha defensin (L12691), ADAM-TS3 (AB002364), cholecystokinin precursor (AW043690), interferon stimulated T-cell alpha chemoattractant precursor (AF030514), and azurocidin (M96326); or any of the following intracellular polypeptides sperminine oxidase (U01134), UDP glycosyltransferase 2 family polypeptide B28 (AF091582), neurotrophic tyrosine kinase receptor 2 (X63759), neutral endopeptidase (J03779), CDC28 protein kinase regulatory subunit 2 (X54942) and beta glucosidase (J03060), lanosterol synthase (U22526), calcium/calmodulin-dependent serine protein kinase (AI688589), estrogen receptor –alternatively spliced transcript H (X86816), chemokine (CX3C motif) receptor 1 (U27699), tyrosinase-related protein 1 (M20681), hydoxy-delta-5-steroid dehydrogenase (AL080151), dihydropyramidinase-like-4 (J03634) and cytochrome P450–family 11 (D84361). Included in this definition are splice variants, isoforms, homologs, degradation products, and fragments of any of the above polypeptides.

By "reference sample" is meant any sample, standard, or level that is used for comparison purposes. A "normal reference sample" can be a prior sample taken from the same subject, a sample from a pregnant subject not having any pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, a sample from a pregnant subject not having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, a subject that is pregnant but the sample was taken early in pregnancy (e.g., in the first or second trimester or before the detection of a pregnancy related hypertensive

disorder, such as pre-eclampsia or eclampsia), a subject that is pregnant and has no history of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, a subject that is not pregnant, a sample of a purified reference polypeptide at a known normal concentration (i.e., not indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia). By "reference standard or level" is meant a value or number derived from a reference sample. A normal reference standard or level can be a value or number derived from a normal subject that is matched to the sample subject by at least one of the following criteria: gestational age of the fetus, maternal age, maternal blood pressure prior to pregnancy, maternal blood pressure during pregnancy, BMI of the mother, weight of the fetus, prior diagnosis of pre-eclampsia or eclampsia, and a family history of pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia. A "positive reference" sample, standard or value is a sample or value or number derived from a subject that is known to have a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, that is matched to the sample subject by at least one of the following criteria: gestational age of the fetus, maternal age, maternal blood pressure prior to pregnancy, maternal blood pressure during pregnancy, BMI of the mother, weight of the fetus, prior diagnosis of a pregnancy related hypertensive disorder, and a family history of a pregnancy related hypertensive disorder

By "reduce or inhibit" is meant the ability to cause an overall decrease preferably of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more in the level of polypeptide or nucleic acid, detected by the aforementioned assays (see "expression") or the biological activity of the polypeptide, detected by the aforementioned assays (see "biological activity"), as compared to a reference sample or a sample not treated with antisense nucleobase oligomers, dsRNA, or siRNA used for RNA interference.

By "sample" is meant a tissue biopsy, cell, bodily fluid (e.g., blood, serum, plasma, urine, saliva, amniotic fluid, or cerebrospinal fluid) or other specimen obtained from a subject. Desirably, the biological sample includes polypeptides of the invention or nucleic acid molecules encoding polypeptides of the invention or both.

By "small interfering RNAs (siRNAs)" is meant a nucleobase oligomer that is preferably a dsRNA molecule, and is preferably greater than 10 nucleotides (nt) in length, more preferably greater than 15 nucleotides in length, and most preferably greater than 19 nucleotides in length that is used to identify the target gene or mRNA to be degraded. Desirably, the siRNA is at least 90%, 95%, 96%, 97%, 98%, 99%, 100% complementary to 18, 19, 20, 21, 22, 23, 24, 25, 35, 45, 50 nucleotides of the desired nucleic acid sequence. A range of 19-25 nucleotides is the most preferred size for siRNAs. siRNAs can also include short hairpin RNA (shRNA) in which both strands of an siRNA duplex are included within a single RNA molecule. siRNA includes any form of dsRNA (proteolytically cleaved products of larger dsRNA, partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA) as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution, and/or alteration of one or more nucleotides. Such alterations can include the addition of non-nucleotide material, such as to the end(s) of the 21 to 23 nt RNA or internally (at one or more nucleotides of the RNA). In a preferred embodiment, the RNA molecules contain a 3' hydroxyl group. Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. Collectively, all such altered RNAs are referred to as analogs of RNA. siRNAs of the present invention need only be sufficiently similar to natural RNA that it has the ability to mediate RNA interference (RNAi). As used herein, RNAi refers to the ATP-dependent targeted cleavage and degradation of a specific mRNA molecule through the

introduction of small interfering RNAs or dsRNAs into a cell or an organism. As used herein "mediate RNAi" refers to the ability to distinguish or identify which RNAs are to be degraded.

By "specifically binds" is meant a compound or antibody which recognizes and binds a polypeptide of the invention but that does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Included in this definition are pregnant, post-partum, and non-pregnant mammals.

By "substantially identical" is meant a nucleic acid or amino acid sequence that, when optimally aligned, for example using the methods described below, share at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a second nucleic acid or amino acid sequence, e.g., an endoglin or soluble endoglin sequence. "Substantial identity" may be used to refer to various types and lengths of sequence, such as full-length sequence, epitopes or immunogenic peptides, functional domains, coding and/or regulatory sequences, exons, introns, promoters, and genomic sequences. Percent identity between two polypeptides or nucleic acid sequences is determined in various ways that are within the skill in the art, for instance, using publicly available computer software such as Smith Waterman Alignment (Smith, T. F. and M. S. Waterman (1981) *J. Mol. Biol.* 147:195-7); "Best Fit" (Smith and Waterman, Advances in Applied Mathematics, 482-489 (1981)) as incorporated into GeneMatcher Plus™, Schwarz and Dayhof (1979) Atlas of Protein Sequence and Structure, Dayhof, M.O., Ed pp 353-358; BLAST program (Basic Local Alignment Search Tool; (Altschul, S. F., W. Gish, et al. (1990) *J. Mol. Biol.* 215: 403-10), BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, or Megalign (DNASTAR) software. In addition, those skilled in

the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the length of the sequences being compared. In general, for proteins, the length of comparison sequences will be at least 6 amino acids, preferably 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, 300, 350, 400, or 500 amino acids or more up to the entire length of the protein. For nucleic acids, the length of comparison sequences will generally be at least 18, 25, 50, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, or at least 1500 nucleotides or more up to the entire length of the nucleic acid molecule. It is understood that for the purposes of determining sequence identity when comparing a DNA sequence to an RNA sequence, a thymine nucleotide is equivalent to a uracil nucleotide. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substrate" or "solid support is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of the nucleic acid probes, polypeptides, or polypeptide binding molecules of the invention and is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible substrates are very large, and include, but are not limited to, glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, Teflon, etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, etc. In general, the substrates allow optical detection and have low background fluorescence.

By "symptoms of pre-eclampsia" is meant any of the following: (1) a systolic blood pressure (BP) >140 mmHg and a diastolic BP >90 mmHg after

20 weeks gestation, (2) new onset proteinuria (1+ by dipstik on urinanaysis, >300mg of protein in a 24 hour urine collection, or random urine protein/creatinine ratio >0.3), and (3) resolution of hypertension and proteinuria by 12 weeks postpartum. The symptoms of pre-eclampsia can also include renal dysfunction and glomerular endotheliosis or hypertrophy. By "symptoms of eclampsia" is meant the development of any of the following symptoms due to pregnancy or the influence of a recent pregnancy: seizures, coma, thrombocytopenia, liver edema, pulmonary edema, and cerebral edema.

By "therapeutic amount" is meant an amount that when administered to a patient suffering from a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, is sufficient to cause a qualitative or quantitative reduction in the symptoms of the pregnancy related hypertensive disorder as described herein. A therapeutic amount can also mean an amount that when administered to a patient suffering from a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, is sufficient to cause a reduction in the expression levels of any one or more of the following: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase. A therapeutic amount can also mean an amount that when administered to a patient suffering from a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, is sufficient to cause an increase in the expression levels of any one or more of the following: alpha defensin,

ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehyrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. Assays for the measurement of the expression levels of polypeptides or a nucleic acid encoding the above polypeptides are known in the art, some of which are described herein.

By "treating" is meant administering a compound or a pharmaceutical composition for prophylactic and/or therapeutic purposes. To "treat disease" or use for "therapeutic treatment" refers to administering treatment to a subject already suffering from a disease to improve the subject's condition. Preferably, the subject is diagnosed as suffering from a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, based on identification of any of the characteristic symptoms described below or the use of the diagnostic methods described herein. To "prevent disease" refers to prophylactic treatment of a subject who is not yet ill, but who is susceptible to, or otherwise at risk of, developing a particular disease. Preferably a subject is determined to be at risk of developing a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, using the diagnostic methods described herein. Thus, in the claims and embodiments, treating is the administration to a mammal either for therapeutic or prophylactic purposes.

By "trophoblast" is meant the mesectodermal cell layer covering the blastocyst that erodes the uterine mucosa and through which the embryo receives nourishment from the mother; the cells contribute to the formation of the placenta.

By "vector" is meant a DNA molecule, usually derived from a plasmid or bacteriophage, into which fragments of DNA may be inserted or cloned. A recombinant vector will contain one or more unique restriction sites, and may

be capable of autonomous replication in a defined host or vehicle organism such that the cloned sequence is reproducible. A vector contains a promoter operably linked to a gene or coding region such that, upon transfection into a recipient cell, an RNA is expressed.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Figure 1 is a graph showing the cumulative distribution function (CDF) for expression ratio greater than 1.0. Software BADGE (Bayesian Analysis of Gene Expression) v1.0 implements a Bayesian approach to identify differentially expressed genes across different experimental conditions. The genes are ranked in order of the conditional probability of increased fold expression given the expression data; the null probability value is 0.5. The ideal CDF has most genes near the null probability value, and few genes have high or low probabilities. For an expected false positive rate of 0.5%, we selected 78 genes, 42 upregulated and 36 downregulated.

Figure 2 is a colormap showing a predictive gene set in normal versus preeclamptic placenta based on mRNA expression using the BADGE program. Rows represent predictive genes for pre-eclampsia while columns represent expression levels for a given patient relative to the average gene expression. The expected false positive rate of 1.0% yields a predictive gene set of 127 genes, with 65 upregulated and 62 downregulated respectively. Significantly upregulated genes include soluble fms-like tyrosine kinase 1 and follistatin-related protein. mRNA expression profile from 3 pre-term placentas are also shown as additional controls.

Figure 3 shows a hierarchical clustering of the affymetrix patient data using Cluster and Treeview, (by Michael Eisen, Stanford University). The samples labeled as P are preeclamptic patients and the samples labeled as N are normal pregnant patients. The dataset was filtered from 12625 to 3564 genes using presence and expression criteria, and the resulting set was median-centered and normalized for genes and arrays. We used hierarchical clustering to analyze possible classes in genes. The cluster includes sFlt1 along with other genes confirmed in literature.

Figure 4 is an autoradiogram showing mRNA expression of Flt-1 and sFlt-1 in pre-eclampsia. mRNA expression of placental sFlt-1 from 3 patients with pre-eclampsia (P1, P2, P3) and three normotensive term pregnancies (N1, N2, N3) were determined by northern blot analysis. The higher band (7.5 kb) is the full length Flt-1 mRNA and the lower, more abundant band (3.4 kb) is the alternatively spliced sFlt-1 mRNA. Actin is included as a control and 28S is shown as arrowhead.

Figure 5 is a set of images showing the immunohistochemistry of Flt-1 expression in normal and preeclamptic placentas. A monoclonal antibody against human Flt-1 was used for these experiments. The data shown here demonstrates increased expression of Flt-1 by the syncitiotrophoblasts of the preeclamptic placenta.

Figure 6A shows the amino acid sequence of follistatin related protein (FLRG) (SEQ ID NO: 1). Figure 6B shows the DNA sequence of follistatin related protein (FLRG) (SEQ ID NO: 2).

Figure 7A shows the amino acid sequence of interleukin 8 (SEQ ID NO: 3). Figure 7B shows the DNA sequence of interleukin 8 (SEQ ID NO: 4).

Figure 8A shows the amino acid sequence of inhibin A (SEQ ID NO: 5). Figure 8B shows the DNA sequence of inhibin A (SEQ ID NO: 6).

Figure 9A shows the amino acid sequence of VEGF-C (SEQ ID NO: 7). Figure 9B shows the DNA sequence of VEGF-C (SEQ ID NO: 8).

Figure 10A shows the amino acid sequence of angiogenin (SEQ ID NO: 9). Figure 10B shows the DNA sequence of angiogenin (SEQ ID NO: 10).

Figure 11A shows the amino acid sequence of beta fertilin (SEQ ID NO: 11). Figure 11B shows the DNA sequence of beta fertilin (SEQ ID NO: 12).

Figure 12 shows the DNA sequence of hypothetical protein (SEQ ID NO: 13).

Figure 13A shows the amino acid sequence of leukocyte associated Ig-like receptor secreted protein (SEQ ID NO: 14). Figure 13B shows the DNA sequence of leukocyte associated Ig-like receptor secreted protein (SEQ ID NO: 15).

Figure 14A shows the amino acid sequence of erythroid differentiation protein (SEQ ID NO: 16). Figure 14B shows the DNA sequence of erythroid differentiation prtoein (SEQ ID NO: 17).

Figure 15A shows the amino acid sequence of adipogenesis inhibitory factor (SEQ ID NO: 18). Figure 15B shows the DNA sequence of adipogenesis inhibitory factor (SEQ ID NO: 19).

Figure 16A shows the amino acid sequence of corticotropin releasing factor binding protein (SEQ ID NO: 20). Figure 16B shows the DNA sequence of corticotropin releasing factor binding protein (SEQ ID NO: 21).

Figure 17A shows the amino acid sequence of alpha-1 anti-chymotrypsin (SEQ ID NO: 22). Figure 17B shows the DNA sequence of alpha-1 anti-chymotrypsin (SEQ ID NO: 23).

Figure 18A shows the amino acid sequence of insulin-like growth factor binding protein-5 (SEQ ID NO: 24). Figure 18B shows the DNA sequence of insulin-like growth factor binding protein-5 (SEQ ID NO: 25).

Figure 19 shows the amino acid sequence of CD33L (SEQ ID NO: 26).

Figure 20A shows the amino acid sequence of cytokine receptor like factor 1 (SEQ ID NO: 27). Figure 20B shows the DNA sequence of cytokine receptor like factor 1 (SEQ ID NO: 28).

Figure 21 shows the amino acid sequence of platelet derived endothelial growth factor (SEQ ID NO: 29).

Figure 22A shows the amino acid sequence of lysyl hydroxylase isoform 2 (SEQ ID NO: 30). Figure 22B shows the DNA sequence of lysyl hydroxylase isoform 2 (SEQ ID NO: 31).

Figure 23A shows the amino acid sequence of stanniocalcin precursor (SEQ ID NO: 32). Figure 23B shows the DNA sequence of stanniocalcin precursor (SEQ ID NO: 33).

Figure 24A shows the amino acid sequence of secreted frizzled related protein (SEQ ID NO: 34). Figure 24B shows the DNA sequence of secreted frizzled related protein (SEQ ID NO: 35).

Figure 25A shows the amino acid sequence of galectin-3 (SEQ ID NO: 36). Figure 25B shows the DNA sequence of galectin-3 (SEQ ID NO: 37).

Figure 26A shows the amino acid sequence of alpha defensin (SEQ ID NO: 38). Figure 26B shows the DNA sequence of alpha defensin (SEQ ID NO: 39).

Figure 27A shows the amino acid sequence of ADAM-TS3 (SEQ ID NO: 40). Figure 27B shows the DNA sequence of ADAM-TS3 (SEQ ID NO: 41).

Figure 28 shows the DNA sequence of cholecystokinin precursor (SEQ ID NO: 42).

Figure 29A shows the amino acid sequence of interferon stimulated T-cell alpha chemoattractant precursor (SEQ ID NO: 43). Figure 29B shows the DNA sequence of interferon stimulated T-cell alpha chemoattractant precursor (SEQ ID NO: 44).

Figure 30A shows the amino acid sequence of azurocidin (SEQ ID NO: 45). Figure 30B shows the DNA sequence of azurocidin (SEQ ID NO: 46).

Figure 31A shows the amino acid sequence of spermine oxidase (SEQ ID NO: 47). Figure 31B shows the DNA sequence of spermine oxidase (SEQ ID NO: 48).

Figure 32A shows the amino acid sequence of UDP glycosyltransferase 2 family polypeptide B28 (SEQ ID NO: 49). Figure 32B shows the DNA sequence of UDP glycosyltransferase 2 family polypeptide B28 (SEQ ID NO: 50).

Figure 33A shows the amino acid sequence of neurotrophic tyrosine kinase receptor 2 (SEQ ID NO: 51). Figure 33B shows the DNA sequence of neurotrophic tyrosine kinase receptor 2 (SEQ ID NO: 52).

Figure 34A shows the amino acid sequence of neutral endopeptidase (SEQ ID NO: 53). Figure 34B shows the DNA sequence of neutral endopeptidase (SEQ ID NO: 54).

Figure 35A shows the amino acid sequence of CDC28 protein kinase regulatory subunit 2 (SEQ ID NO: 55). Figure 35B shows the DNA sequence of CDC28 protein kinase regulatory subunit 2 (SEQ ID NO: 56).

Figure 36 shows the DNA sequence of beta glucosidase (SEQ ID NO: 57).

Figure 37A shows the amino acid sequence of lanosterol synthase (SEQ ID NO: 58). Figure 37B shows the DNA sequence of lanosterol synthase (SEQ ID NO: 59).

Figure 38 shows the DNA sequence of calcium/calmodulin-dependent serine protein kinase (SEQ ID NO: 60).

Figure 39 shows the DNA sequence of estrogen receptor-alternatively spliced transcript H (SEQ ID NO: 61).

Figure 40A shows the amino acid sequence of chemokine (CX3C motif) receptor 1 (SEQ ID NO: 62). Figure 40B shows the DNA sequence of chemokine (CX3C motif) receptor 1 (SEQ ID NO: 63).

Figure 41A shows the amino acid sequence of tyrosinase-related protein 1 (SEQ ID NO: 64). Figure 41B shows the DNA sequence of tyrosinase-related protein 1 (SEQ ID NO: 65).

Figure 42 shows the DNA sequence of hydroxy-delta-5-steroid dehydrogenase (SEQ ID NO: 66).

Figure 43A shows the amino acid sequence of dihydropyramidinase-like-4 (SEQ ID NO: 67). Figure 43B shows the DNA sequence of dihydropyramidinase-like-4 (SEQ ID NO: 68).

Figure 44 shows the amino acid sequence of cytochrome P450 family 11 (SEQ ID NO: 69).

Detailed Description

In order to identify secreted factors involved in the pathogenesis of pregnancy related hypertensive disorders, such as pre-eclampsia, we performed gene expression profiling of placental tissue from 19 women with pre-eclampsia and 15 nomotensive pregnant women using Affymetrix U95A microarray chips. Data were analyzed using the computer program BADGE (Bayesian Analysis of Differential Gene Expression version 1.0) (http://genomethods.org/badge) (see Ramoni and Sebastiani, in Berthold and Hand eds. *Intelligent Data Analysis: An Introduction*, Springer, New York,NY (1999)) and hierarchical clustering analysis (Eisen et al., *Proc. Natl. Acad. Sci.*, 95:14863-8 (1998)) to identify differentially expressed genes across experimental conditions. We discovered that the gene encoding the following secreted polypeptides showed increased expression in blood samples taken from women with pre-eclampsia: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived

endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3. We also discovered that expression levels of the genes encoding the following secreted polypeptides were decreased in blood samples taken from women with pre-eclampsia: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin. In addition, we also discovered that genes encoding the following intracellular polypeptides or enzymes showed increased expression in placentas from women with pre-eclampsia: sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase. Genes encoding the following intracellular gene polypeptides showed decreased expression in placentas from women with pre-eclampsia: lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11.

For the purposes of the descriptions below, all of the polypeptides described above are collectively referred to as "the polypeptides of the invention." While the detailed description presented herein refers specifically to polypeptides associated with specific GenBank accession numbers, it will be clear to one skilled in the art that the detailed description can also apply to family members, isoforms, homologs, fragments, and/or variants or the specified polypeptides.

We have also discovered therapeutic agents that reduce the expression or biological activity of any one or more of the following polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-

chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin -3, or agents that increase the expression levels or biological activity of any one or more of the following polypeptides: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, or azurocidin, can be used to treat or prevent pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia in a subject. Such agents include, but are not limited to, antibodies, nucleobase oligomers for antisense or RNAi, purified natural or synthetic compounds, chemical compounds, and small molecules.

The invention also features methods for measuring levels of any one or more of the polypeptides of the invention or a nucleic acid encoding a polypeptide of the invention as a detection tool for early diagnosis and management of pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia.

Diagnostics

The present invention features assays based on the detection of at least one of the polypeptides of the invention to diagnose pregnancy related hypertensive disorders, such as pre-eclampsia, eclampsia, or the propensity to develop such conditions. The present invention also features diagnostic assays based on the detection of at least two, at least three, at least four, or at least five or more polypeptides of the invention to diagnose pregnancy related hypertensive disorders, such as pre-eclampsia, eclampsia, or a predisposition to such conditions. Levels of any one or more of the polypeptides of the invention (either free, bound, or total levels) are measured in a subject sample and used as an indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia, eclampsia, or a predisposition to such conditions. The diagnostic methods can also be combined with methods to detect levels of any

additional markers of pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, such as soluble endoglin, sFlt-1, VEGF, or PlGF. In one embodiment, a metric incorporating the levels of any one or more of the polypeptides of the invention, soluble endoglin, sFlt-1, VEGF, or PlGF, or any combination thereof, is used to determine whether a relationship between levels of at least two of the polypeptides is indicative of pre-eclampsia or eclampsia.

Standard methods may be used to measure levels of any one or more of the polypeptides of the invention in any bodily fluid, including, but not limited to, urine, blood, serum, plasma, saliva, amniotic fluid, or cerebrospinal fluid. Such methods include immunoassay, ELISA, western blotting using antibodies directed to the polypeptide of the invention and quantitative enzyme immunoassay techniques such as those described in Ong et al. (*Obstet. Gynecol.* 98:608-611, 2001) and Su et al. (*Obstet. Gynecol.*, 97:898-904, 2001). ELISA assays are the preferred method for measuring levels of a polypeptide of the invention. In preferred embodiments, the level of follistatin related protein, inhibin-A, beta fertilin, or insulin-like growth factor binding protein –5 is measured. In additional preferred embodiments, the body mass index (BMI) and gestational age of the fetus is also measured and included the diagnostic metric. For example, if the level of any of the following polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3 is increased (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more), relative to a reference sample, this is considered a positive indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In another example, if the levels of any one of the

following proteins: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin is decreased (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more), relative to a reference sample, this is considered a positive indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

Metrics measuing the levels of sFlt-1, VEGF, PlGF, and/or soluble endoglin can also be used in combination with any of the diagnostic methods of the invention. For example, the PAAI (sFlt-1/ VEGF + PlGF) is used, in combination with measurement of any one or more polypeptides of the invention, as an anti-angiogenic index that is diagnostic of pregnancy related hypertensive disorders, such as pre-eclampsia, eclampsia, or the propensity to develop such conditions. The PAAI (sFlt-1/ VEGF + PlGF) ratio is merely one example of a useful metric that may be used as a diagnostic indicator. It is not intended to limit the invention. Another example is the following soluble endoglin anti-angiogenic index: (sFlt-1 + 0.25(soluble endoglin polypeptide))/PlGF. Virtually any metric that detects an alteration in the levels of any polypeptide of the invention, soluble endoglin, sFlt-1, PlGF, or VEGF, or any combination thereof, in a subject relative to a reference sample may be used as a diagnostic indicator. One example of a metric that can be used in the diagnostic methods of the invention is (sFlt1+soluble endoglin+follistatin related protein)/PlGF.

Expression levels of particular nucleic acids or polypeptides may be correlated with a particular disease state (e.g., pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia), and thus are useful in diagnosis. Oligonucleotides or longer fragments derived from a nucleic acid sequence encoding a polypeptide of the invention may be used as a probe not only to monitor expression, but also to identify subjects having a genetic variation, mutation, or polymorphism in a nucleic acid molecule, encoding a polypeptide of the invention, that is indicative of a predisposition to develop the conditions.

These polymorphisms may affect nucleic acid or polypeptide expression levels or biological activity. Detection of genetic variation, mutation, or polymorphism relative to a normal, reference sample can be used as a diagnostic indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia, eclampsia, or a predisposition to develop such disorders.

Such genetic alterations may be present in the promoter sequence, an open reading frame, intronic sequence, or untranslated 3' region of a gene. Information related to genetic alterations can be used to diagnose a subject as having a pregnancy related hypertensive disorder, such as pre-eclampsia, eclampsia, or a predisposition to develop such conditions. As noted throughout, specific alterations in the levels of biological activity of any polypeptide of the invention or any combination thereof, can be correlated with the likelihood of developing a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or the predisposition to the same. As a result, one skilled in the art, having detected a given mutation, can then assay one or more of the biological activities of the polypeptide to determine if the mutation causes or increases the likelihood of pre-eclampsia or eclampsia.

In one embodiment, a subject having pre-eclampsia, eclampsia, or a propensity to develop such conditions will show an alteration in the expression of a nucleic acid encoding a polypeptide of the invention. Methods for detecting such alterations in nucleic acids are standard in the art and are described in Ausubel et al., *supra*. In one example northern blotting or real-time PCR is used to detect mRNA levels for a nucleic acid encoding any polypeptide of the invention.

In another embodiment, hybridization with PCR probes that are capable of detecting a nucleic acid molecule encoding a polypeptide of the invention, including genomic sequences, or closely related molecules, may be used to hybridize to a nucleic acid sequence derived from a subject having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or at risk of developing such conditions. The specificity of the probe, whether it is made

from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), determine whether the probe hybridizes to a naturally occurring sequence, allelic variants, or other related sequences. Hybridization techniques may be used to identify mutations indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or may be used to monitor expression levels of a gene encoding a polypeptide of the invention (for example, by Northern analysis, Ausubel et al., *supra*).

A subject having a pregnancy related hypertensive disorder, such as pre-eclampsia, eclampsia, or a propensity to develop such conditions will show an increase relative to a reference sample or level (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) in the expression of a secreted or intracellular polypeptide or a nucleic acid encoding a secreted or intracellular polypeptide selected from the group consisting of: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase, relative to a reference sample. In another example, a subject having a pregnancy related hypertensive disorder, such as pre-eclampsia, eclampsia, or a propensity to develop such conditions will show a decrease (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) relative to a reference sample or level in the expression of

a secreted or intracellular polypeptide or a nucleic acid encoding a secreted or intracellular polypeptide selected from the group consisting of: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11, relative to a reference sample.

A variety of protocols for measuring an alteration in the expression of such polypeptides are known, including immunological methods (such as ELISAs and RIAs), and provide a basis for diagnosing a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a risk of developing such conditions.

In one embodiment, the level of at least one polypeptide or nucleic acid encoding a polypeptide of the invention is measured in combination with the level of soluble endoglin, sFlt-1, VEGF, or PlGF polypeptide or nucleic acid, or any combination thereof. Methods for the measurement of sFlt-1, VEGF, PlGF, and soluble endoglin are described in U.S. Patent Application Publication Numbers U.S. Patent Application Publication Numbers 20040126828, 20050025762, and 20050170444; PCT Publication Numbers WO 2004/008946 and WO 2005/077007; and U.S Patent Application Serial Number 11/235,577, each of which is hereby incorporated by reference in its entirety.

In one example, the measurement of any of the nucleic acids or polypeptides described herein preferably occurs on at least two different occasions and an alteration in the levels over time is used as an indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or the propensity to develop such conditions. In another example, the measurement of any of the nucleic acids or polypeptides described herein is compared to a reference sample and an alteration as compared to normal

reference levels is used as an indicator of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or the propensity to develop such conditions.

The level of any polypeptide of the invention in the bodily fluids of a subject having pre-eclampsia, eclampsia, or the propensity to develop such conditions may be altered by as little as 10%, 20%, 30%, or 40%, or by as much as 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more relative to the level of the same polypeptide in a reference sample. The level of any polypeptide of the invention in the bodily fluids of a subject having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or the propensity to develop such conditions may be altered by as little as 10%, 20%, 30%, or 40%, or by as much as 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more over time from one measurement to the next.

In one embodiment, a subject sample of a bodily fluid (e.g., urine, plasma, serum, amniotic fluid, or cerebrospinal fluid) is collected early in pregnancy prior to the onset of symptoms of the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. In another example, the sample can be a tissue or cell collected early in pregnancy prior to the onset of symptoms of the pregnancy related hypertensive disorder. Non-limiting examples include placental tissue, placental cells, endothelial cells, and leukocytes such as monocytes. In humans, for example, maternal blood serum samples are collected from the antecubital vein of pregnant women during the first, second, or third trimesters of the pregnancy. Preferably, the assay is carried out during the first trimester, for example, at 4, 6, 8, 10, or 12 weeks, or during the second trimester, for example at 14, 16, 18, 20, 22, or 24 weeks. Such assays may also be conducted at the end of the second trimester or the third trimester, for example at 26, 28, 30, 32, 34, 36, 38, or 40 weeks. It is preferable that levels of one or more polypeptides of the invention be measured twice during this period of time. For the diagnosis of post-partum pre-eclampsia or eclampsia, the assay is carried out postpartum. For the diagnosis

of a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, the assay may be carried out prior to the onset of pregnancy. In one example, for the monitoring and management of therapy, the assay is carried out after the diagnosis of pre-eclampsia but during the pregnancy.

In one particular example, a sample of bodily fluid (e.g., (blood, serum, plasma, urine, amniotic fluid, and cerebrospinal fluid) is collected during pregnancy and the levels of at least one polypeptide of the invention determined by ELISA. In another example, a sample is collected during the second trimester and early in the third trimester and in increase or decrease in the level of a polypeptide of the invention from the first sampling to the next is indicative of pre-eclampsia or eclampsia, or the propensity to develop either. In another particular example, serial blood samples can be collected during pregnancy and the levels of any one or more of the polypeptides of the invention determined by ELISA. In another example, a sample is collected during the second trimester and early in the third trimester and an alteration in the levels of any one or more of the polypeptides of the invention from the first sampling to the next is indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a predisposition thereto.

In veterinary practice, assays may be carried out at any time during the pregnancy but are preferably carried out early in pregnancy, prior to the onset of symptoms of the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. Given that the term of pregnancies varies widely between species, the timing of the assay will be determined by a veterinarian, but will generally correspond to the timing of assays during a human pregnancy.

The diagnostic methods described herein can be used individually or in combination with any other diagnostic method described herein for a more accurate diagnosis of the presence of, severity of, or estimated time of onset of the pregnancy related hypertensive disorder, such as pre-eclampsia or

eclampsia. For example, the diagnostic methods using the nucleic acids that encode the polypeptides of the invention can be used initially and then increased expression of the polypeptide can be confirmed using standard immunological methods (e.g., western blooting or ELISA). In addition, the diagnostic methods described herein can be used in combination with any other diagnostic methods determined to be useful for the accurate diagnosis of the presence of, severity of, or estimated time of onset of the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. The diagnostic methods described herein can also be used to monitor and manage pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia in a subject.

Expression level of each polypeptide or nucleic acids encoding polypeptides of the invention may be considered individually, although it is within the scope of the invention to provide combinations of two or more polypeptides of the invention or nucleic acids encoding polypeptides of the invention for use in the methods and compositions of the invention to increase the confidence of the analysis. A panel comprises two or more polypeptides of the invention, or fragments thereof, two or more, 2-5, 5-10, 10-15, 15-20, 20-25 or more than 25 nucleic acid molecules, or fragments thereof or complementary nucleic acid molecules, or two or more binding molecules, such as antibodies, that recognize a polypeptide of the invention. In one embodiment, these panels of polypeptides of the invention are selected such that the polypeptides of the invention within any one panel share certain features, such as polypeptides that are shown herein to be increased in samples from pre-eclamptic women. Similarly, different panels of polypeptides of the invention may be composed of polypeptides of the invention representing different stages of a pregnancy related hypertensive disorder, for example separate panels for mild-pre-eclampsia, to severe pre-eclampsia, to eclampsia.

Panels of the polypeptides of the invention can also include binding molecules (e.g., antibodies) that specifically bind sFlt-1, VEGF, PlGF, and soluble endoglin, and may further be provided on biochips, as discussed below.

Diagnostic Kits

The invention also provides for a diagnostic test kit. The diagnostic test kit includes the components or reagents required to carry out any of the diagnostic assays described above and instructions for the use of the components or reagents to diagnose a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a predisposition to a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. For example, a diagnostic test kit can include antibodies to any polypeptide of the invention and components required to detect, and more preferably to evaluate, binding between the antibodies and the polypeptide of the invention. Non-lmiting examples of antibodies useful in the diagnostic methods and kits of the invention include human FLRG antibody, catalog number AF1288, R&D systems, Minneapolis, MN and human secreted frizzled related protein antibody, catalog number AF1384, R&D systems, Minneapolis, MN. For detection, either the antibody or the polypeptide of the invention is labeled, and either the antibody or the polypeptide of the invention is substrate-bound, such that polypeptide of the invention-antibody interaction can be established by determining the amount of label attached to the substrate following binding between the antibody and the polypeptide of the invention. A conventional ELISA is a common, art-known method for detecting antibody-substrate interaction and can be provided with the kit of the invention. Polypeptides of the invention can be detected in virtually any bodily fluid including, but not limited to urine, serum, plasma, saliva, amniotic fluid, or cerebrospinal fluid. The invention also provides for a diagnostic test kit that includes a nucleic acid encoding a polypeptide of the invention that can be used to detect and determine levels of nucleic acids encoding a polypeptide of the invention. A

kit that determines an alteration in the level of a polypeptide of the invention relative to a reference, such as the level present in a normal control, is useful as a diagnostic kit in the methods of the invention.

The diagnostic kits of the invention can also include antibodies or nucleic acids for the detection of soluble endoglin, sFlt-1, VEGF, or PlGF polypeptides or nucleic acids as described in U.S. Patent Application Publication Numbers 20040126828, 20050025762, and 20050170444; PCT Publication Numbers WO 2004/008946 and WO 2005/077007; and U.S. Patent Application Serial Number 11/235,577.

Desirably, the kit includes any of the components needed to perform any of the diagnostic methods described above. In one embodiment of the invention, such a kit includes a solid support (e.g., a membrane or a microtiter plate) coated with a primary agent (e.g., an antibody or protein that recognizes the antigen), standard solutions of purified protein for preparation of a standard curve, a body fluid (e.g. serum or urine) control for quality testing of the analytical run, a secondary agent (e.g., a second antibody reactive with a second epitope in the antigen to be detected or an antibody or protein that recognizes the primary antibody) conjugated to a label or an enzyme such as horse radish peroxidase or otherwise labeled, a substrate solution, a stopping solution, a washing buffer and an instruction manual. The membrane can be supported on a dipstick structure where the sample is deposited on the membrane by placing the dipstick structure into the sample or the membrane can be supported in a lateral flow cassette where the sample is deposited on the membrane through an opening in the cassette. The kit can also be in an array format and can include an array of polypeptides of the invention or binding molecules that specifically bind polypeptides of the invention arranged on a biochip, such as, for example, a GeneChip™.

The diagnostic kits also generally include a label or instructions for the intended use of the kit components and a reference sample or purified proteins to be used to establish a standard curve. In one example, the kit contains

instructions for the use of the kit for the diagnosis of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or the propensity to develop pre-eclampsia or eclampsia. In yet another example, the kit contains instructions for the use of the kit to monitor therapeutic treatment or dosage regimens for the treatment of pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia. It will be understood that the reference sample values will depend on the intended use of the kit. For example, the sample can be compared to a normal reference value, wherein an alteration in the levels of one or more of the polypeptides of the invention or a metric using levels of one or more of the polypeptides of the invention is indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a predisposition to pre-eclampsia or eclampsia. In another example, a kit used for therapeutic monitoring can have a reference value that is indicative of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, wherein an alteration in the level of one or more of the polypeptides of the invention or a metric using levels of one or more of the polypeptides of the invention relative to the reference sample can be used to indicate therapeutic efficacy or effective dosages of therapeutic compounds.

Arrays and Biochips

The invention also includes an array comprising a panel of polypeptides of the invention. The array can be used to assay expression of one or more genes or polypeptides in the array.

It will be appreciated by one skilled in the art that the panels of polypeptides of the invention of the invention may be provided on solid supports, as a biochip. For example, polynucleotides may be coupled to an array (e.g., a biochip using GeneChip™ for hybridization analysis), to a resin (e.g., a resin which can be packed into a column for column chromatography), or a matrix (e.g., a nitrocellulose matrix for northern blot analysis). The immobilization of nucleic acid molecules complementary to nucleic acid

molecules encoding any of the polypeptides of the invention, either covalently or noncovalently, permits a discrete analysis of the presence or activity of each of the nucleic acid molecules encoding the polypeptides of the invention in a sample. In an array, for example, polynucleotides complementary to each member of a panel of nucleic acid molecules encoding polypeptides of the invention may individually be attached to different, known locations on the array. The array may be hybridized with, for example, polynucleotides extracted from a bodily fluid, tissue, or cell sample from a subject. The hybridization of polynucleotides from the sample with the array at any location on the array can be detected, and thus the presence or quantity of the nucleic acids or transcripts encoding polypeptides of the invention in the sample can be ascertained. In one embodiment, an array based on a biochip is employed. Similarly, immunological analyses may be performed using protein arrays or antibody arrays that include immobilized antibodies or other binding molecules specific for polypeptides of the invention. Such protein arrays can be hybridized with a bodily fluid, tissue, or cell sample, which contains polypeptides of the invention or antibodies to polypeptides of the invention, from a subject. Additional details on examples of arrays and biochips can be found, for example, in U.S. Patent Application Publication No. 20050266409, herein incorporated by reference.

Exemplary binding molecules and antibodies

Examples of antibodies and binding proteins that can be used in the diagnostic methods and kits of the invention are described below. The antibodies described below can also be used in the therapeutic methods of the invention and can be modified to increase potency or stability or to reduce reactivity to the antibodies. These examples are intended to illustrate the invention and not to limit the invention in anyway.

Follistatin related protein

Follistatin related protein, also known as FLRG, FSRP, FRP, FLS-1, and FSTL1, is a protein related to follistatin. Follistatin is a secreted glycoprotein that binds activin in vitro and in vivo and inhibits the biological functions of activin. Follistatin related protein also binds to activin with high affinity and is expressed in the basement membrane between the dermis and the epidermis and around blood vessels. The gene encoding follistatin related protein, FLRG, was induced during the wound healing process (Wankell et al., *J. Endocrin.* 171:385-395 (2001) and Tortoriello et al., *Endocrinology* 142:3426-3434 (2001)).

Activin and other TGFβ superfamily members, or fragments thereof, can be used as specific binding molecules to detect follistatin related protein in a biological sample. Exemplary antibodies that specifically bind follistatin related protein that can also be used to detect follistatin related protein in a biological sample include the polyclonal FSRP antibody described in Tortorielle et al., *supra*, and antibodies available from Abnova Corporation (e.g., catalog no. H00010468-A01) and human FLRG antibody, R&D systems (e.g., catalog nos. AF1288 and AF1694).

Inhibin A

Inhibin is a disulfide-linked, dimeric glycoprotein composed of an α-subunit and one of two β-subunits. Inhibin is a member of the TGFβ superfamily and is expressed in the adrenal cortex. One hypothesis regarding inhibin action is that inhibin binds the membrane bound serine-threonine kinase ActRII subunit, and blocks the signal generating subunit (ActRI) phosphorylation, thereby antagonizing activin activation. One example of a protein that specifically binds to inhibin A is betaglycan (Vale et al., *Ann. N. Y. Acad. Sci.* 1038:142-147 (2004). Betaglycan, or fragments thereof, can be used as specific binding molecules to detect follistatin related protein in a biological sample. Examples of antibodies, or antigen binding fragments thereof, that

specifically bind inhibin A that can also be used to detect inhibin A in a biological sample include antibodies available from Abnova Corp. (e.g., catalog no. H00003624-A01), Abcam (e.g., catalog no. Ab10599, Ab724), and Genetex (e.g., catalog no. GTX10599 and GTX20724), and the antibody described in Rishi et al., *Am. J. Surg. Pathol.* 21:583-589 (1997).

Beta fertilin

Beta fertilin, also known as fertilin beta, is a sperm protein that is a candidate molecule fro mediating the binding and fusión of the sperm and egg plasma membranes. Fertilin is a heterodimer with a beta subunit that has a region of homology to the disintegrin family of integrin ligands and an alpha subunit that has a region of homology to viral fusion peptides. Fertilin alpha and beta have also been shown to interact with the heat shock protein calmegin. (Ikawa et al., *Dev. Biol.* 240:254-261 (2001) and Evans et al., *Dev. Biol.* 187:94-106 (1997)).

Calmegin, or fragments thereof, can be used as specific binding molecules to detect beta fertilin in a biological sample. Examples of antibodies, or antigen binding fragments thereof, that specifically bind beta fertilin that can also be used to detect beta fertilin in a biological sample include the antibodies described in Ikawa et al., supra, and antibodies commercially available from Chemicon (e.g., catalog nos. MAB 19292 and 19030) and United States Biological (e.g., catalog no. A0858-070).

Insulin like growth factor binding protein-5

Insulin like growth factor binding protein-5, also known as IGFBP-5 or ILGFBP-5, is a member of the superfamily of insulin-like growth factor binding proteins, which are cysteine-rich proteins with conserved cysteine residues clustered in the amino-terminal and the carboxy-terminal regions of the molecule. IGFBP-5 interacts with IGF-I and functions to inhibit the survival effect of IGF-I (Tonner et al., *Development* 129:4547-4557 (2002))

and modulate IGF-I ligand-receptor interactions (Tonner et al., *Adv. Exp. Med. Biol.* 480:45-53 (2000)). Additional IGFBP-5 binding proteins include plasminogen activator inhibitor-1 (Tonner et al., *J. Endocrinol.* 167:265-73 (2000)) and alphas2-casein (Tonner et al., *Adv. Exp. Med. Biol.* 480:45-53 (2000)).

IGF, plasminogen activator inhibitor-1, alpha s2-casein, or any fragments thereof, can be used as specific binding molecules to detect IGFBP-5 in a biological sample. Examples of antibodies, or antigen binding fragments thereof, that specifically bind IGFBP-5 that can also be used to detect IGFBP-5 in a biological sample include the antibodies from Diagnostic Systems Laboratories Inc. (e.g., catalog no. R00737), Alpha Diagnostic International (e.g., catalog no. IGFBP5-1s) and Abcam (e.g., catalog no. Ab4257).

Secreted frizzled related protein

The secreted frizzled related proteins are a family of secreted proteins that contain an N-terminal signal peptide, a frizzled-related CRD, and a C-terminal hydrophilic region with some homology to the netrins, but lack evidence of any transmembrane domains.

The secreted frizzled related proteins appear to act as soluble modulators of Wnt signaling, presumably by competing with membrane frizzled receptors for the binding of secreted Wnt ligands.

Any Wnt family member protein, or any fragments thereof, can be used as specific binding molecules to detect secreted frizzled related protein in a biological sample. One example of an antibody that specifically binds secreted frizzled related protein and can be used to detect secreted frizzled related protein in a biological sample is the human secreted frizzled related protein antibody, (catalog no. AF1384) from R&D systems.

Screening Assays

As discussed above, the expression level of one ore more polypeptides of the invention or nucleic acids encoding a polypeptide of the invention is altered in a subject having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a propensity to develop such conditions. Based on these discoveries, polypeptides of the invention (both intracellular and secreted) are useful for the high-throughput low-cost screening of candidate compounds to identify those that modulate the expression of a polypeptide of the invention or nucleic acid molecule encoding a polypeptide of the invention whose expression is altered in a subject having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

Any number of methods are available for carrying out screening assays to identify new candidate compounds that alter the expression of a nucleic acid molecule encoding a polypeptide of the invention. In one working example, candidate compounds are added at varying concentrations to the culture medium of cultured cells expressing a nucleic acid sequence encoding a polypeptide of the invention. Exemplary cell cultures include any mammalian, yeast, insect, or bacterial cell cultures. Preferred cell cultures include mammalian cell cultures such as trophoblasts (e.g., BEWO, JAR, and JEG cells) and HUVECs. These cells can then be used to screen for new candidate compounds. Gene expression is then measured, for example, by microarray analysis, Northern blot analysis (Ausubel et al., *supra*), or RT-PCR, using any appropriate fragment prepared from the nucleic acid molecule as a hybridization probe. The level of gene expression in the presence of the candidate compound is compared to the level measured in a control culture medium lacking the candidate compound. A compound considered to be useful in the invention is one that promotes a decrease in the expression of a polypeptide, or fragment thereof, or a nucleic acid molecule encoding the polypeptide, or fragment thereof, selected from the following group of polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C,

angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta-glucosidase. Additional useful compounds are compounds that promote an increase in the expression of a polypeptide, or fragment thereof, or a nucleic acid molecule encoding the polypeptide, or fragment thereof, selected from the following group of polypeptides: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin, or the level of any one of the following intracellular polypeptides, or fragments thereof, in a sample from the subject: lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11. Such compounds may be used, for example, as a therapeutic to treat pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, in a subject.

In another working example, the effect of candidate compounds may be measured at the level of polypeptide production using the same general approach and standard immunological techniques, such as Western blotting or immunoprecipitation with an antibody specific for a polypeptide of the invention. For example, immunoassays may be used to detect or monitor the expression of at least one of the polypeptides of the invention in an organism. Polyclonal or monoclonal antibodies (produced as described above) that are capable of binding to such a polypeptide may be used in any standard

immunoassay format (e.g., ELISA, western blot, or RIA assay) to measure the level of the polypeptide. In some embodiments, a compound that promotes a decrease in the expression or biological activity of a polypeptide of the invention is considered particularly useful. Again, such a molecule may be used, for example, as a therapeutic to delay, ameliorate, or treat the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or the symptoms of the pregnancy related hypertensive disorder in a subject.

In yet another working example, candidate compounds may be screened to identify those that specifically bind to a polypeptide of the invention. The efficacy of such a candidate compound is dependent upon its ability to interact with such a polypeptide or a functional equivalent thereof. Such an interaction can be readily assayed using any number of standard binding techniques and functional assays (e.g., those described in Ausubel et al., *supra*). In one embodiment, a candidate compound may be tested *in vitro* for its ability to specifically bind a polypeptide of the invention.

In another working example, a nucleic acid encoding a polypeptide of the invention is expressed as a transcriptional or translational fusion protein with a detectable reporter, and expressed in an isolated cell (e.g., mammalian or insect cell) under the control of a heterologous promoter, such as an inducible promoter. The cell expressing the fusion protein is then contacted with a candidate compound, and the expression of the detectable reporter in that cell is compared to the expression of the detectable reporter in an untreated control cell. A candidate compound that alters (e.g., increases or decreases) the expression of a polypeptide of the invention fused to a detectable reporter is a compound that is useful for the treatment of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia.

In one particular working example, a candidate compound that binds to a polypeptide of the invention may be identified using a chromatography-based technique. For example, a recombinant polypeptide of the invention may be purified by standard techniques from cells engineered to express the

polypeptide (e.g., those described above) and may be immobilized on a column. A solution of candidate compounds is then passed through the column, and a compound specific for the immobilized polypeptide of the invention is identified on the basis of its ability to bind to the polypeptide and be immobilized on the column. To isolate the compound, the column is washed to remove non-specifically bound molecules, and the compound of interest is then released from the column and collected. Similar methods may be used to isolate a compound bound to a polypeptide microarray. Compounds isolated by this method (or any other appropriate method) may, if desired, be further purified (e.g., by high performance liquid chromatography). In addition, these candidate compounds may be tested for their ability to alter (e.g., increase or decrease) the activity of a polypeptide of the invention. Compounds isolated by this approach may also be used, for example, as therapeutics to treat a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, in a human subject. Compounds that are identified as binding to a polypeptide of the invention with an affinity constant less than or equal to 10 mM are considered particularly useful in the invention. Alternatively, any *in vivo* protein interaction detection system, for example, any two-hybrid assay may be utilized to identify compounds or proteins that bind to a polypeptide of the invention.

Potential antagonists include organic molecules, peptides, peptide mimetics, polypeptides, nucleic acids, and antibodies that bind to a polypeptide of the invention or a nucleic acid sequence encoding a polypeptide of the invention.

DNA sequences encoding a polypeptide of the invention may also be used in the discovery and development of a therapeutic compound for the treatment of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia. The encoded polypeptide, upon expression, can be used as a target for the screening of drugs. Additionally, the DNA sequences encoding

the amino terminal regions of the encoded polypeptide or Shine-Delgarno or other translation facilitating sequences may be isolated by standard techniques (Ausubel et al., *supra*).

Optionally, compounds identified in any of the above-described assays may be confirmed as useful in an assay for compounds that alter (e.g., increase or decrease) the biological activity of a polypeptide of the invention using standard assays such as those described herein.

Small molecules of the invention preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is preferred that these small molecules are organic molecules.

Test compounds and extracts

In general, compounds capable of altering (e.g., increasing or decreasing) the activity of a polypeptide of the invention are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries or from polypeptide or nucleic acid libraries, according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Compounds used in screens may include known compounds (for example, known therapeutics used for other diseases or disorders). Alternatively, virtually any number of unknown chemical extracts or compounds can be screened using the methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates

(Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their molt-disrupting activity should be employed whenever possible.

When a crude extract is found to alter (e.g., increase or decrease) the activity of a polypeptide of the invention by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more, or to bind to a polypeptide of the invention, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract that alters (e.g., increases or decreases) the activity of a polypeptide of the invention. Methods of fractionation and purification of such heterogeneous extracts are known in the art. If desired, compounds shown to be useful as therapeutics for the treatment of a pregnancy related hypertensive disorder in a human are chemically modified according to methods known in the art.

Therapeutics

The present invention features methods and compositions for treating or preventing pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, in a subject. We have discovered that levels of follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3 are increased in subjects having pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, or predisposition thereto. Therefore, the invention includes methods and agents that decrease the expression levels or biological activity of any one or more of these polypeptides or nucleic acid molecules. Such agents include compounds that downregulate or inhibit the biological activity of any one or more of the above polypeptides; a purified antibody or antigen-binding fragment that specifically binds any one of the above polypeptides; antisense nucleobase oligomers; and dsRNAs targeting any of the above polypeptides. These methods are described in detail below.

We have also discovered that the levels of alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin are decreased in subjects having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a predisposition to develop such conditions. Therefore, the invention also includes any methods and agents that increase the expression levels or biological activity of any one or more of these polypeptides or nucleic acid molecules. Such agents include

compounds that upregulate or increase the biological activity of any one or more of the above polypeptides or purified forms of the polypeptides themselves.

These methods and agents can be combined with any additional therapies for pregnancy related hypertensive disorders such as therapeutics aimed at decreasing sFlt-1 or soluble endoglin levels or increasing VEGF or PlGF levels as described in U.S. Patent Application Publication Numbers 20040126828, 20050025762, and 20050170444; PCT Publication Numbers WO 2004/008946 and WO 2005/077007; and U.S Patent Application Serial Number 11/235,577.

In addition to the use of compounds that can increase the levels of any of the above polypeptides in a subject sample, the invention provides for the use of any chronic hypertension medications used in combination with any of the therapeutic methods described herein. Medications used for the treatment of hypertension during pregnancy include methyldopa, hydralazine hydrochloride, or labetalol. For each of these medications, modes of administration and dosages are determined by the physician and by the manufacturer's instructions.

Purified proteins

In a preferred embodiment of the present invention, purified forms of any one or more of the following polypeptides: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin are administered to the subject in order to treat or prevent pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia.

Purified alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin polypeptides include any polypeptide with an amino acid sequence that is homologous, more desirably, substantially identical to the amino acid sequence

of alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin, that can induce angiogenesis or that is capable of promoting selective growth of vascular endothelial cells or umbilical vein endothelial cells.

Therapeutic nucleic acids

Recent work has shown that the delivery of nucleic acid molecules (e.g., DNA or RNA) capable of expressing an endothelial cell mitogen such as VEGF to the site of a blood vessel injury will induce proliferation and reendothelialization of the injured vessel. While the present invention does not relate to blood vessel injury, these general techniques for the delivery of nucleic acid to endothelial cells can be used in the present invention for the delivery of nucleic acids encoding alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, or azurocidin. These general techniques are described in U.S. Patent Nos. 5,830,879 and 6,258,787 and are incorporated herein by reference.

In the present invention, the nucleic acid molecule may be any nucleic acid (e.g., DNA or RNA) including genomic DNA, cDNA, and mRNA, encoding any of the following: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, or azurocidin. The nucleic acids encoding the desired protein may be obtained using routine procedures in the art, e.g. recombinant DNA, PCR amplification.

Modes for delivering nucleic acids

For any of the nucleic acid applications described herein, standard methods for administering nucleic acids can be used. For example, to simplify the manipulation and handling of the nucleic acid encoding any of the following polypeptides: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, or azurocidin; the nucleic acid is preferably inserted into a cassette where it is

operably linked to a promoter. The promoter must be capable of driving expression of the polypeptide in the desired target host cell. The selection of appropriate promoters can readily be accomplished. Preferably, one would use a high expression promoter. An example of a suitable promoter is the 763-base-pair cytomegalovirus (CMV) promoter. The Rous sarcoma virus (RSV) (Davis, et al., *Hum. Gene Ther.* 4:151-159, 1993) and mouse mammary tumor virus (MMTV) promoters may also be used. Certain proteins can be expressed using their native promoter. Other elements that can enhance expression can also be included (e.g., enhancers or a system that results in high levels of expression such as a tat gene and tar element). The recombinant vector can be a plasmid vector such as pUC118, pBR322, or other known plasmid vectors, that includes, for example, an *E. coli* origin of replication (see, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory press, 1989). The plasmid vector may also include a selectable marker such as the β lactamase gene for ampicillin resistance, provided that the marker polypeptide does not adversely affect the metabolism of the organism being treated. The cassette can also be bound to a nucleic acid binding moiety in a synthetic delivery system, such as the system disclosed in PCT Publication No. WO95/22618.

The nucleic acid can be introduced into the cells by any means appropriate for the vector employed. Many such methods are well known in the art (Sambrook et al., *supra*, and Watson et al., "Recombinant DNA", Chapter 12, 2d edition, Scientific American Books, 1992). Recombinant vectors can be transferred by methods such as calcium phosphate precipitation, electroporation, liposome-mediated transfection, gene gun, microinjection, viral capsid-mediated transfer, polybrene-mediated transfer, or protoplast fusion. For a review of the procedures for liposome preparation, targeting and delivery of contents, see Mannino and Gould-Fogerite, (*Bio Techniques*, 6:682-690, 1988), Felgner and Holm, (*Bethesda Res. Lab. Focus*, 11:21, 1989) and Maurer (*Bethesda Res. Lab. Focus,* 11:25, 1989).

Transfer of the recombinant vector (either plasmid vector or viral vectors) can be accomplished through direct injection into the amniotic fluid or intravenous delivery.

Gene delivery using adenoviral vectors or adeno-associated vectors (AAV) can also be used. Adenoviruses are present in a large number of animal species, are not very pathogenic, and can replicate equally well in dividing and quiescent cells. As a general rule, adenoviruses used for gene delivery are lacking one or more genes required for viral replication. Replication-defective recombinant adenoviral vectors used for the delivery of a nucleic acid encoding a desired protein, can be produced in accordance with art-known techniques (see Quantin et al., *Proc. Natl. Acad. Sci. USA*, 89:2581-2584, 1992; Stratford-Perricadet et al., *J. Clin. Invest.*, 90:626-630, 1992; and Rosenfeld et al., *Cell*, 68:143-155, 1992). For an example of the use of gene therapy *in utero* see U.S. Patent No. 6,399,585.

Once transferred, the nucleic acid is expressed by the cells at the site of injury for a period of time sufficient to increase blood serum levels of the desired protein. Because the vectors containing the nucleic acid are not normally incorporated into the genome of the cells, expression of the protein of interest takes place for only a limited time. Typically, the protein is expressed at therapeutic levels for about two days to several weeks, preferably for about one to two weeks. Re-application of the DNA can be utilized to provide additional periods of expression of the therapeutic protein.

Therapeutic nucleobase oligomers that inhibit protein expression

The present invention also features the use of nucleobase oligomers to downregulate expression of any of the following: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth

factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3.

In one example, the nucleobase oligomer is an antisense nucleobase oligomer. By binding to the complementary nucleic acid sequence (the sense or coding strand), antisense nucleobase oligomers are able to inhibit protein expression presumably through the enzymatic cleavage of the RNA strand by RNAse H. Preferably the antisense nucleobase oligomer is capable of reducing expression of one or more of the above polypeptides or nucleic acids encoding one or more of the above polypeptides in a cell that expresses increased levels of that protein. Preferably the decrease in protein expression is at least 10% relative to cells treated with a control nucleobase oligomer, more preferably 25%, and most preferably 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater. Methods for selecting and preparing antisense nucleobase oligomers are well known in the art. For an example of the use of antisense nucleobase oligomers to downregulate VEGF expression see U.S. Patent No. 6,410,322. Methods for assaying levels of protein expression are also well known in the art and include western blotting, immunoprecipitation, and ELISA.

The present invention also features the use of RNA interference (RNAi) to inhibit expression of any one or more of the following: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3. RNA interference (RNAi) is a mechanism of post-transcriptional gene silencing (PTGS) in which double-stranded RNA (dsRNA)

corresponding to a gene or mRNA of interest is introduced into an organism resulting in the degradation of the corresponding mRNA. In the RNAi reaction, both the sense and anti-sense strands of a dsRNA molecule are processed into small RNA fragments or segments ranging in length from 18 to 25 nucleotides, preferably 21 to 23 nucleotides (nt), and having 2-nucleotide 3' tails. Alternatively, synthetic dsRNAs, which are 21 to 23 nt in length and have 2-nucleotide 3' tails, can be synthesized, purified and used in the reaction. These 21 to 23 nt dsRNAs are known as "guide RNAs" or "short interfering RNAs" (siRNAs). dsRNAs or siRNAs that are useful in the present invention are substantially complementary (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) to at least 18, 19, 20, 21, 22, 23, 24, or 25 consecutive nucleotides of a gene encoding any one or more of the following polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3.

The siRNA duplexes then bind to a nuclease complex composed of proteins that target and destroy endogenous mRNAs having homology to the siRNA within the complex. Although the identity of the proteins within the complex remains unclear, the function of the complex is to target the homologous mRNA molecule through base pairing interactions between one of the siRNA strands and the endogenous mRNA. The mRNA is then cleaved approximately 12 nt from the 3' terminus of the siRNA and degraded. In this manner, specific genes can be targeted and degraded, thereby resulting in a loss

of protein expression from the targeted gene. siRNAs can also be chemically synthesized or obtained from a company that chemically synthesizes siRNAs (e.g., Dharmacon Research Inc., Pharmacia, or ABI).

General descriptions of the specific requirements and modifications of dsRNA are described in PCT Publication No. WO01/75164. While dsRNA molecules can vary in length, it is most preferable to use siRNA molecules which are 21- to 23- nucleotide dsRNAs with characteristic 2- to 3- nucleotide 3' overhanging ends typically either (2'-deoxy) thymidine or uracil. The siRNAs typically comprise a 3' hydroxyl group. Single stranded siRNA as well as blunt ended forms of dsRNA and shRNA can also be used. In order to further enhance the stability of the RNA, the 3' overhangs can be stabilized against degradation. In one such embodiment, the RNA is stabilized by including purine nucleotides, such as adenosine or guanosine. Alternatively, substitution of pyrimidine nucleotides by modified analogs, e.g., substitution of uridine 2-nucleotide overhangs by (2'-deoxy) thymidine is tolerated and does not affect the efficiency of RNAi. The absence of a 2' hydroxyl group significantly enhances the nuclease resistance of the overhang in tissue culture medium.

Alternatively siRNA can be prepared using any of the methods set forth in PCT Publication No. WO01/75164 or using standard procedures for *in vitro* transcription of RNA and dsRNA annealing procedures as described in Elbashir et al. (*Genes & Dev.,* 15:188-200, 2001). siRNAs are also obtained as described in Elbashir et al. by incubation of dsRNA that corresponds to a sequence of the target gene in a cell-free Drosophila lysate from syncytial blastoderm Drosophila embryos under conditions in which the dsRNA is processed to generate siRNAs of about 21 to about 23 nucleotides, which are then isolated using techniques known to those of skill in the art. For example, gel electrophoresis can be used to separate the 21-23 nt RNAs and the RNAs

can then be eluted from the gel slices. In addition, chromatography (e.g., size exclusion chromatography), glycerol gradient centrifugation, and affinity purification with antibody can be used to isolate the 21 to 23 nt RNAs.

A variety of methods are available for transfection, or introduction, of dsRNA or oligonucleotides into mammalian cells. For example, there are several commercially available transfection reagents including but not limited to: TransIT-TKO™ (Mirus, Cat. # MIR 2150), Transmessenger™ (Qiagen, Cat. # 301525), and Oligofectamine™ (Invitrogen, Cat. # MIR 12252-011). Protocols for each transfection reagent are available from the manufacturer.

In the present invention, the dsRNA, or siRNA, is substantially complementary (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) to at least a portion of the mRNA sequence of any one of the following proteins: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin –3 and can reduce or inhibit the expression of the protein. Preferably, the decrease in protein expression is at least 10% relative to cells treated with a control dsRNA or siRNA, more preferably 25%, and most preferably at least 50%. Methods for assaying levels of protein expression are also well known in the art and include western blotting, immunoprecipitation, and ELISA.

In the present invention, the nucleobase oligomers used include any modification that enhances the stability or function of the nucleic acid in any way. Examples include modifications to the phosphate backbone, the internucleotide linkage, or to the sugar moiety. Examples of modifications that may be used in the nucleobase oligomers of the invention, can be found in U.S.

Patent Application Publication Nos. 20030114412, paragraphs [0030] to [0046] and 20030114407, paragraphs [0036] to [0055], and 20030190659, paragraphs [0083] to [0106].

Assays for gene and protein expression

The following methods can be used to evaluate protein or gene expression and determine efficacy for any of the above-mentioned methods for increasing or decreasing the expression of any one or more polypeptides of the invention.

A sample from the subject (e.g., a bodily fluid such as blood, serum, plasma, urine, amniotic fluid, and cerebrospinal fluid, a cell, or a tissue) is measured for levels of a desired polypeptide, using methods such as ELISA, western blotting, or immunoassays using specific antibodies. Methods used to measure serum levels of polypeptides include ELISA, western blotting, or immunoassays using specific antibodies. A positive result is considered an alteration of at least 20%, preferably 30%, more preferably at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more in the serum levels of a polypeptide of the invention as compared to a reference sample.

In addition, *in vitro* angiogenesis assays can be performed to determine if the subject's blood has converted from an anti-angiogenic state to a pro-angiogenic state. One example of such an *in vitro* assay for angiogenesis is the endothelial tube assay. In this assay, growth factor reduced Matrigel (7 mg/mL, Collaborative Biomedical Products, Bedford, MA) is placed in wells (100 µl/well) of a pre-chilled 48-well cell culture plate and is incubated at 37° C for 25-30 minutes to allow polymerization. Human umbilical vein endothelial cells (30,000 + in 300 µl of endothelial basal medium with no serum, Clonetics, Walkersville, MD) at passages 3-5 are treated with 10% patient serum, plated onto the Matrigel coated wells, and are incubated at 37° C for 12-16 hours. Tube formation is then assessed through an inverted phase contrast microscope at 4X (Nikon Corporation, Tokyo, Japan) and is analyzed

(tube area and total length) using the Simple PCI imaging analysis software. A positive result can be considered conversion from an anti-angiogenic state to a pro-angiogenic state using the *in vitro* angiogenesis assay.

Bodily fluid samples from the subject can also be measured for levels of nucleic acid encoding a polypeptide of the invention. There are several art-known methods to assay for gene expression. Some examples include the preparation of RNA from the blood samples of the subject and the use of the RNA for northern blotting, PCR based amplification, or RNAse protection assays.

Use of antibodies for therapeutic treatment

The use of compounds, such as antibodies, to bind to and neutralize the activity of any one or more of the following polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein -5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin –3, can be used to prevent or treat pre-eclampsia or eclampsia.

The present invention provides antibodies that bind specifically to the any of the above proteins. The antibodies are used to neutralize the activity of any one or more of the above proteins. Methods for the preparation and use of antibodies for therapeutic purposes are described in several patents including U.S. Patent Numbers 6,054,297; 5,821,337; 6,365,157; and 6,165,464 and are incorporated herein by reference. Antibodies can be polyclonal or monoclonal;

monoclonal antibodies are preferred. Some examples of antibodies to some of the polypeptides of the invention are described above under "Exemplary binding molecules and antibodies."

Monoclonal antibodies, particularly those derived from rodents including mice, have been used for the treatment of various diseases; however, there are limitations to their use including the induction of a human anti-mouse immunoglobulin response that causes rapid clearance and a reduction in the efficacy of the treatment. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller et al., *Blood*, 62:988-995 1983; Schroff et al., *Cancer Res.*, 45:879-885, 1985).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (U.S. Pat. No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA,* 81:6851-6855, 1984; Boulianne et al., *Nature,* 312:643-646, 1984; Neuberger et al., *Nature,* 314:268-270, 1985). The production and use of such chimeric antibodies are described below.

A cocktail of the monoclonal antibodies of the present invention can be used as an effective treatment for pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia. The cocktail may include as few as two, three, or four different antibodies or as many as six, eight, or ten different antibodies. In addition, the antibodies of the present invention can be combined with an anti-hypertensive drug (e.g., methyldopa, hydralazine hydrochloride, or labetalol) or any other medication used to treat pregnancy related hypertensive disorders, such as pre-eclampsia or eclampsia, or the symptoms associated with pregnancy related hypertensive disorders.

Non-limiting examples of antibodies that are useful in the methods of the invention are as follows: anti-interleukin 8 (see Leong et al. *Cytokine* 16:106-119, 2001 and Mian et al., *Clin. Cancer Res.* 9:3167-3175, 2003); anti-

inhibin A (Verotec Catalog No. MCA951S, see Rishi et al. *Am. J. Surg. Pathol.* 21:582-589, 1997); and anti-VEFG-C (e.g., Alitalo et al., U.S. Patent No. 6,361,946).

Preparation of Antibodies

Monoclonal antibodies that specifically bind to any of the polypeptides of the invention may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein (*Nature,* 256: 495-497, 1975) and Campbell ("Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam, 1985), as well as by the recombinant DNA method described by Huse et al. (*Science*, 246, 1275-1281, 1989).

Monoclonal antibodies may be prepared from supernatants of cultured hybridoma cells or from ascites induced by intra-peritoneal inoculation of hybridoma cells into mice. The hybridoma technique described originally by Kohler and Milstein (*Eur. J. Immunol*, 6, 511-519, 1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

The route and schedule of immunization of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. Typically, mice are used as the test model, however, any mammalian subject including human subjects or antibody producing cells therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

After immunization, immune lymphoid cells are fused with myeloma cells to generate a hybrid cell line that can be cultivated and subcultivated indefinitely, to produce large quantities of monoclonal antibodies. For

purposes of this invention, the immune lymphoid cells selected for fusion are lymphocytes and their normal differentiated progeny, taken either from lymph node tissue or spleen tissue from immunized animals. The use of spleen cells is preferred, since they offer a more concentrated and convenient source of antibody producing cells with respect to the mouse system. The myeloma cells provide the basis for continuous propagation of the fused hybrid. Myeloma cells are tumor cells derived from plasma cells. Murine myeloma cell lines can be obtained, for example, from the American Type Culture Collection (ATCC; Manassas, VA). Human myeloma and mouse-human heteromyeloma cell lines have also been described (Kozbor et al., *J. Immunol.*, 133:3001-3005, 1984; Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, pp. 51-63, 1987).

The hybrid cell lines can be maintained *in vitro* in cell culture media. Once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media such as hypoxanthine-aminopterin-thymidine (HAT) medium. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromatography, affinity chromatography, or the like.

The antibody may be prepared in any mammal, including mice, rats, rabbits, goats, and humans. The antibody may be a member of one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof, and preferably is an IgG antibody.

While the preferred animal for producing monoclonal antibodies is mouse, the invention is not so limited; in fact, human antibodies may be used and may prove to be preferable. Such antibodies can be obtained by using human hybridomas (Cole et al., "Monoclonal Antibodies and Cancer Therapy",

Alan R. Liss Inc., p. 77-96, 1985). In the present invention, techniques developed for the production of chimeric antibodies by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule can be used (Morrison et al., *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger et al., *Nature* 312, 604-608, 1984; Takeda et al., *Nature* 314, 452-454, 1985); such antibodies are within the scope of this invention and are described below.

As another alternative to the cell fusion technique, Epstein-Barr virus (EBV) immortalized B cells are used to produce the monoclonal antibodies of the present invention (Crawford D. et al., *J. of Gen. Virol.,* 64:697-700, 1983; Kozbor and Roder, *J. Immunol.*, 4:1275-1280, 1981; Kozbor et al., *Methods in Enzymology,* 121:120-140, 1986). In general, the procedure consists of isolating Epstein-Barr virus from a suitable source, generally an infected cell line, and exposing the target antibody secreting cells to supernatants containing the virus. The cells are washed, and cultured in an appropriate cell culture medium. Subsequently, virally transformed cells present in the cell culture can be identified by the presence of the Epstein-Barr viral nuclear antigen, and transformed antibody secreting cells can be identified using standard methods known in the art. Other methods for producing monoclonal antibodies, such as recombinant DNA, are also included within the scope of the invention.

Preparation of immunogens

Any of the polypeptides of the invention may be used alone as an immunogen, or may be attached to a carrier protein or to other objects, such as sepharose beads. Any of the proteins of the invention may be purified from cells known to express the endogenous protein such as human umbilical vein endothelial cells (trophoblasts or HUVEC; Burrows et al., *Clin. Cancer Res.* 1:1623-1634, 1995; Fonsatti et al., *Clin. Cancer Res.* 6:2037-2043, 2000). Additionally, nucleic acid molecules that encode any of the polypeptides of the invention, or portions thereof, can be inserted into known vectors for

expression in host cells using standard recombinant DNA techniques. Suitable host cells for protein expression include baculovirus cells (e.g., Sf9 cells), bacterial cells (e.g., *E. coli*), and mammalian cells (e.g., NIH3T3 cells).

In addition, peptides derived from any of the polypeptides of the invention can be synthesized and used as immunogens. The methods for making antibody to peptides are well known in the art and generally require coupling the peptide to a suitable carrier molecule, such as serum albumin. Peptides can be any length, preferably 10 amino acids or greater, more preferably 25 amino acids or greater, and most preferably 40, 50, 60, 70, 80, or 100 amino acids or greater. Preferably, the amino acid sequences are at least 60%, more preferably 85%, and, most preferably 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of any of the nucleic acid sequences encoding the polypeptides of the invention. The peptides can be commercially obtained or made using techniques well known in the art, such as, for example, the Merrifield solid-phase method (*Science*, 232:341-347, 1985). The procedure may use commercially available synthesizers such as a Biosearth 9500 automated peptide machine, with cleavage of the blocked amino acids being achieved with hydrogen fluoride, and the peptides purified by preparative HPLC using a Waters Delta Prep 3000 instrument, on a 15-20 μm Vydac C4 PrepPAK column.

Functional equivalents of antibodies

The invention also includes functional equivalents of the antibodies described in this specification. Functional equivalents include polypeptides with amino acid sequences substantially identical to the amino acid sequence of the variable or hypervariable regions of the antibodies of the invention. Functional equivalents have binding characteristics comparable to those of the antibodies, and include, for example, chimerized, humanized and single chain antibodies as well as fragments thereof. Methods of producing such functional equivalents are disclosed, for example, in PCT Publication No. WO93/21319;

European Patent Application No. 239,400; PCT Publication No. WO89/09622; European Patent Application No. 338,745; European Patent Application No. 332424; and U.S. Patent No. 4,816,567; each of which is herein incorporated by reference.

Chimerized antibodies preferably have constant regions derived substantially or exclusively from human antibody constant regions and variable regions derived substantially or exclusively from the sequence of the variable region from a mammal other than a human. Such humanized antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Methods for humanizing non-human antibodies are well known in the art (for reviews see Vaswani and Hamilton, *Ann Allergy Asthma Immunol.,* 81:105-119, 1998 and Carter, *Nature Reviews Cancer*, 1:118-129, 2001). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the methods known in the art (Jones et al., *Nature*, 321:522-525, 1986; Riechmann et al., *Nature,* 332:323-329, 1988; and Verhoeyen et al., *Science*, 239:1534-1536 1988), by substituting rodent CDRs or other CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species (see for example, U.S. Patent No. 4,816,567). In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies (Presta, *Curr. Op. Struct. Biol.,* 2:593-596, 1992).

Additional methods for the preparation of humanized antibodies can be found in U.S. Patent Nos. 5,821,337, 6,054,297, 6,639,055, and Carter, (*supra*) which are all incorporated herein by reference. The humanized antibody is selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3, and IgG_4. Where cytotoxic activity is not needed, such as in the present invention, the constant domain is preferably of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Marks et al., *J. Mol. Biol.,* 222:581-597, 1991 and Winter et al. *Annu. Rev. Immunol.,* 12:433-455, 1994). The techniques of Cole et al. and Boerner et al. are also useful for the preparation of human monoclonal antibodies (Cole et al., *supra*; Boerner et al., *J. Immunol.,* 147: 86-95, 1991).

Suitable mammals other than a human include any mammal from which monoclonal antibodies may be made. Examples of mammals other than a human include, for example a rabbit, rat, mouse, horse, goat, or primate; a mouse is preferred.

Functional equivalents of antibodies also include single-chain antibody fragments, also known as single-chain antibodies (scFvs). Single-chain antibody fragments are recombinant polypeptides which typically bind antigens or receptors; these fragments contain at least one fragment of an antibody variable heavy-chain amino acid sequence (V_H) tethered to at least one fragment of an antibody variable light-chain sequence (V_L) with or without one or more interconnecting linkers. Such a linker may be a short, flexible peptide selected to assure that the proper three-dimensional folding of the V_L and V_H domains occurs once they are linked so as to maintain the target molecule binding-specificity of the whole antibody from which the single-chain antibody fragment is derived. Generally, the carboxyl terminus of the V_L or V_H

sequence is covalently linked by such a peptide linker to the amino acid terminus of a complementary V_L and V_H sequence. Single-chain antibody fragments can be generated by molecular cloning, antibody phage display library or similar techniques. These proteins can be produced either in eukaryotic cells or prokaryotic cells, including bacteria.

Single-chain antibody fragments contain amino acid sequences having at least one of the variable regions or CDRs of the whole antibodies described in this specification, but are lacking some or all of the constant domains of those antibodies. These constant domains are not necessary for antigen binding, but constitute a major portion of the structure of whole antibodies. Single-chain antibody fragments may therefore overcome some of the problems associated with the use of antibodies containing part or all of a constant domain. For example, single-chain antibody fragments tend to be free of undesired interactions between biological molecules and the heavy-chain constant region, or other unwanted biological activity. Additionally, single-chain antibody fragments are considerably smaller than whole antibodies and may therefore have greater capillary permeability than whole antibodies, allowing single-chain antibody fragments to localize and bind to target antigen-binding sites more efficiently. Also, antibody fragments can be produced on a relatively large scale in prokaryotic cells, thus facilitating their production. Furthermore, the relatively small size of single-chain antibody fragments makes them less likely than whole antibodies to provoke an immune response in a recipient.

Functional equivalents further include fragments of antibodies that have the same or comparable binding characteristics to those of the whole antibody. Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably the antibody fragments contain all six CDRs of the whole antibody, although fragments containing fewer than all of such regions, such as three, four or five CDRs, are also functional.

Further, the functional equivalents may be or may combine members of any one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof.

Preparation of Functional Equivalents of Antibodies

Equivalents of antibodies are prepared by methods known in the art. For example, fragments of antibodies may be prepared enzymatically from whole antibodies. Preferably, equivalents of antibodies are prepared from DNA encoding such equivalents. DNA encoding fragments of antibodies may be prepared by deleting all but the desired portion of the DNA that encodes the full-length antibody.

DNA encoding chimerized antibodies may be prepared by recombining DNA substantially or exclusively encoding human constant regions and DNA encoding variable regions derived substantially or exclusively from the sequence of the variable region of a mammal other than a human. DNA encoding humanized antibodies may be prepared by recombining DNA encoding constant regions and variable regions other than the CDRs derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived substantially or exclusively from a mammal other than a human.

Suitable sources of DNA molecules that encode fragments of antibodies include cells, such as hybridomas, that express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into equivalents, as described above.

The DNA deletions and recombinations described in this section may be carried out by known methods, such as those described in the published patent applications listed above.

Antibody Screening and Selection

Monoclonal antibodies are isolated and purified using standard art-known methods. For example, antibodies can be screened using standard art-known methods such as ELISA or western blot analysis. Non-limiting examples of such techniques are described in Examples II and III of U.S. Patent No. 6,365,157, herein incorporated by reference.

Therapeutic Uses of Antibodies

When used *in vivo* for the treatment or prevention of a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, the antibodies of the subject invention are administered to the subject in therapeutically effective amounts. Preferably, the antibodies are administered parenterally or intravenously by continuous infusion. The dose and dosage regimen depends upon the severity of the disease, and the overall health of the subject. The amount of antibody administered is typically in the range of about 0.001 to about 10 mg/kg of subject weight, preferably 0.01 to about 5 mg/kg of subject weight.

For parenteral administration, the antibodies are formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate may also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies typically are formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Combination therapies

Optionally, a therapeutic of the invention may be administered in combination with any other standard pregnancy related hypertensive disorder therapeutic; such methods are known to the skilled artisan.

Dosages and Modes of Administration

Preferably, the therapeutic compound of the invention is administered during pregnancy for the treatment or prevention of the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or after pregnancy to treat post-partum pre-eclampsia or eclampsia. Techniques and dosages for administration vary depending on the type of compound (e.g., chemical compound, antibody, antisense, or nucleic acid vector) and are well known to those skilled in the art or are readily determined.

Therapeutic compounds of the present invention may be administered with a pharmaceutically acceptable diluent, carrier, or excipient, in unit dosage form. Administration may be parenteral, intravenous, subcutaneous, oral or local by direct injection into the amniotic fluid. Intravenous delivery by continuous infusion is the preferred method for administering the therapeutic compounds of the present invention.

The composition can be in the form of a pill, tablet, capsule, liquid, or sustained release tablet for oral administration; or a liquid for intravenous, subcutaneous or parenteral administration; or a polymer or other sustained release vehicle for local administration.

Methods well known in the art for making formulations are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A.R. Gennaro AR., 2000, Lippincott Williams & Wilkins, Philadelphia, PA). Formulations for parenteral administration may, for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or

polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Nanoparticulate formulations (e.g., biodegradable nanoparticles, solid lipid nanoparticles, liposomes) may be used to control the biodistribution of the compounds. Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The concentration of the compound in the formulation varies depending upon a number of factors, including the dosage of the drug to be administered, and the route of administration.

The compound may be optionally administered as a pharmaceutically acceptable salt, such as non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose and sorbitol), lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc).

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium.

The dosage and the timing of administering the compound depends on various clinical factors including the overall health of the subject and the

severity of the symptoms of the pregnancy related hypertensive disorder, such as pre-eclampsia. In general, once the pregnancy related hypertensive disorder, such as pre-eclampsia or a propensity to develop pre-eclampsia, is detected, continuous infusion of the purified protein is used to treat or prevent further progression of the condition. Treatment can be continued for a period of time ranging from 1 to 100 days, more preferably 1 to 60 days, and most preferably 1 to 20 days, or until the completion of pregnancy. Dosages vary depending on each compound and the severity of the condition and are titrated to achieve a steady-state blood serum concentration.

Subject monitoring

The diagnostic methods described herein can also be used to monitor the pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, during therapy or to determine the dosages of therapeutic compounds. In one example, a therapeutic compound is administered and the level of expression of a polypeptide of the invention is determined during the course of therapy.

Therapeutics that modulate the expression of any one or more nucleic acids or polypeptides of the invention are taken as particularly useful in the invention.

In one example, a therapeutic agent or method that decreases, by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more, the level of any of the following polypeptides or nucleic acids encoding the polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2

family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase during the course of therapy, is considered to be an effective therapeutic agent or an effective dosage of a therapeutic agent. In another example, a therapeutic agent or method that increases, by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more, the level of any of the following polypeptides or nucleic acids encoding the polypeptides: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11 during the course of therapy, is considered to be an effective therapeutic agent or an effective dosage of a therapeutic agent.

The disease state or treatment of a subject having a pregnancy related hypertensive disorder, such as pre-eclampsia or eclampsia, or a propensity to develop such a condition, can be monitored using the methods and compositions of the invention. In one embodiment, the expression of a polypeptide of the invention present in a bodily fluid, such as urine, plasma, amniotic fluid, or CSF, is monitored. Such monitoring may be useful, for example, in assessing the efficacy of a particular drug in a subject or in assessing disease progression.

Examples

Example 1. Gene expression profiling of placental tissue from pre-eclamptic and normotensive women.

In order to identify novel secreted factors involved in the pathogenesis of pre-eclampsia, we performed gene expression profiling of placental tissue from 19 women with pre-eclampsia and 15 nomotensive pregnant women using Affymetrix U95A microarray chips (see Table 1).

Table 1: Clinical characteristics of the study patients

	Normal (n=15)	Pre-eclampsia (n=19)
Maternal Age (years)	35.2	31.9
Gestational Age (wks)	39.0	31.1*
Primiparous (%)	19	81*
Systolic BP (mm Hg)	107	167.2**
Diastolic BP (mm Hg)	83	101.8**
Proteinuria (g protein/g creat)	<0.3	5.2**
Serum Uric Acid (mg/dl)	NA	6.8
Hematocrit (%)	35.7	33.9
Platelet Count (K/μl)	217	198
Serum Creatinine (mg/dl)	0.5	0.6

Data shown are mean values. * $p<0.05$, ** $p<0.005$

Data were analyzed using the computer program BADGE (Bayesian Analysis of Differential Gene Expression version 1.0) (http://genomethods.org/badge) (see Ramoni and Sebastiani, in Berthold and Hand eds. *Intelligent Data Analysis: An Introduction*, Springer, New York,NY (1999)) and hierarchical clustering analysis (Eisen et al., *Proc. Natl. Acad. Sci.*, 95:14863-8 (1998)) to identify differentially expressed genes across experimental conditions (Figure 1). The software BADGE (Bayesian Analysis of Gene Expression) v1.0 implements a Bayesian approach to identify differentially expressed genes across different experimental conditions.

Cumulative distribution function (CDF) for expression ratio greater than 1.0. The genes are ranked in order of the conditional probability of increased fold expression given the expression data; the null probability value is 0.5.

A predictive gene set in normal versus pre-eclampsia placenta mRNA expression was discovered using the BADGE program. A colormap of the predictive gene set is shown in Figure 2. Rows represent predictive genes for pre-eclampsia while columns represent expression levels for a given patient relative to the average gene expression. The expected false positive rate of 1.0% yields a predictive gene set of 127 genes, with 65 upregulated and 62 downregulated respectively (Table 2). (See Figures 6A – 44 for amino acid and nucleic acid sequences for the polypeptides of the invention).

Table 2. Summary of predictive genes

Affy Probe	Genbank	Probability	Fold	Gene Symbol	Gene Name
33900_at	U76702	0.99992	3.849	FSTL3	follistatin-like 3 (secreted glycoprotein)
990_at	X51602	0.99990	3.233	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
991_g_at	X51602	0.99989	2.727	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
1601_s_at	M11567	0.99986	3.254	IGFBP5	insulin-like growth factor binding protein 5
36317_at	U57057	0.99982	3.767	CORO2A	coronin, actin binding protein, 2A
1389_at	J03779	0.99982	2.299	MME	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)
501_g_at	U37143	0.99980	2.293	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2
37657_at	Y16270	0.99979	3.089	PALM	paralemmin
HUMGAPDH	L27559	0.99978	3.647	GAPD	glyceraldehyde-3-phosphate dehydrogenase
159_at	U61836	0.99969	3.343	VEGFC	vascular endothelial growth factor C
31754_at	AI950015	0.99966	3.737	ABCA12	ATP-binding cassette, sub-family A (ABC1), member 12
1149_at	D16154	0.99960	3.241	---	Transcription Factor Eb
1545_g_at	U43142	0.99959	2.692	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
34129_at	D86358	0.99953	2.211	STXBP5L	syntaxin binding protein 5-like
1103_at	HG4740	0.99952	3.141	ANG	angiogenin, ribonuclease, RNase A family, 5

Affy Probe	Genbank	Probability	Fold	Gene Symbol	Gene Name
255_s_at	X52009	0.99950	2.761	INHA	inhibin, alpha
1650_g_at	U01134	0.99948	2.745	SMOX	spermine oxidase
1964_g_at	M74297	0.99946	2.331	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
32298_at	L35848	0.99940	2.894	ADAM2	a disintegrin and metalloproteinase domain 2 (fertilin beta)
33995_at	M77144	0.99939	5.997	GUCA2A	guanylate cyclase activator 2A (guanylin)
32892_at	AF058989	0.99937	2.014	RPS6KA2	ribosomal protein S6 kinase, 90kDa, polypeptide 2
41577_at	W27723	0.99910	2.361	PPP1R16B	protein phosphatase 1, regulatory (inhibitor) subunit 16B
40790_at	X53004	0.99903	2.169	BHLHB2	basic helix-loop-helix domain containing, class B, 2
41024_f_at	AF055033	0.99891	2.617	GYPE	glycophorin E
36426_g_at	AF052095	0.99879	1.981	NEBL	nebulette
34800_at	L37362	0.99868	2.943	LRIG1	leucine-rich repeats and immunoglobulin-like domains 1
36979_at	L26953	0.99868	2.389	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
31382_f_at	AF091582	0.99851	2.065	UGT2B28	UDP glycosyltransferase 2 family, polypeptide B28
40357_at	U20350	0.99831	3.380	INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)
1963_at	U01134	0.99822	2.714	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
35865_at	AB001915	0.99815	2.632	NR5A2	nuclear receptor subfamily 5, group A, member 2
39051_at	X86400	0.99814	1.805	NNAT	neuronatin
33642_s_at	X68733	0.99807	3.236	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
33182_at	X63759	0.99804	2.698	NTRK2	neurotrophic tyrosine kinase, receptor, type 2
33639_g_at	U17986	0.99802	1.694	ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
34483_at	AL039458	0.99793	2.234	SIGLEC6	sialic acid binding Ig-like lectin 6
1511_at	S77812	0.99793	1.771	SHC3	src homology 2 domain containing transforming protein C3
38280_s_at	U43753	0.99787	3.286	NTRK2	neurotrophic tyrosine kinase, receptor, type 2
41420_at	AB020630	0.99785	2.479	IGFBP5	insulin-like growth factor binding protein 5
34088_at	AB023223	0.99783	2.009	NXPH4	neurexophilin 4
36284_at	Y17673	0.99781	2.978	LY6D	lymphocyte antigen 6 complex, locus D
33825_at	M97496	0.99777	2.575	SERPINA3	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
36533_at	D83402	0.99742	2.354	PTGIS	prostaglandin I2 (prostacyclin) synthase

Affy Probe	Genbank	Probability	Fold	Gene Symbol	Gene Name
37813_at	AL079273	0.99735	2.073	DDX51	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51
39202_at	W26403	0.99731	1.667	TRAF3IP1	TNF receptor-associated factor 3 interacting protein 1
368_at	Z29083	0.99721	1.904	TPBG	trophoblast glycoprotein
500_at	U37143	0.99716	1.751	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2
38078_at	AF042166	0.99699	1.774	FLNB	filamin B, beta (actin binding protein 278)
41608_at	X58022	0.99693	2.906	CRHBP	corticotropin releasing hormone binding protein
1734_at	M60556	0.99656	2.200	---	Human transforming growth factor beta-3 gene, 5 end
1945_at	M25753	0.99644	1.747	CCNB1	cyclin B1
31990_at	AF009624	0.99636	1.496	KIF17	kinesin family member 17
36933_at	D87953	0.99618	2.050	NDRG1	N-myc downstream regulated gene 1
32562_at	X72012	0.99610	1.941	ENG	endoglin (Osler-Rendu-Weber syndrome 1)
32565_at	U66619	0.99606	2.098	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3
1369_s_at	M28130	0.99601	3.111	IL8	interleukin 8
1678_g_at	M65062	0.99589	2.334	IGFBP5	insulin-like growth factor binding protein 5
37887_at	AF086904	0.99572	1.887	CHEK2	CHK2 checkpoint homolog (S. pombe)
40690_at	X54942	0.99568	1.913	CKS2	CDC28 protein kinase regulatory subunit 2
40926_at	U52111	0.99559	2.068	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
34898_at	M30704	0.99558	2.179	AREG	amphiregulin (schwannoma-derived growth factor)
33748_at	D86976	0.99546	2.523	HA-1	minor histocompatibility antigen HA-1
35940_at	X64624	0.99536	2.086	POU4F1	POU domain, class 4, transcription factor 1
32632_g_at	J03060	0.99526	2.108	GBAP	glucosidase, beta; acid, pseudogene
33792_at	AF043498	0.99518	2.318	PSCA	prostate stem cell antigen
38566_at	X60382	0.00495	0.730	COL10A1	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)
31740_s_at	AB008913	0.00488	0.637	PAX4	paired box gene 4
33359_at	AB018311	0.00485	0.547	LPHN3	latrophilin 3
38519_at	U68233	0.00476	0.483	NR1H4	nuclear receptor subfamily 1, group H, member 4
33046_f_at	X68879	0.00473	0.492	EMX1	empty spiracles homolog 1 (Drosophila)
39108_at	U22526	0.00472	0.616	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
33693_at	M76482	0.00451	0.499	DSG3	desmoglein 3 (pemphigus vulgaris antigen)
834_at	U40462	0.00436	0.615	ZNFN1A1	zinc finger protein, subfamily 1A, 1 (Ikaros)
34575_f_at	U10689	0.00416	0.480	MAGEA5	melanoma antigen, family A, 5

Affy Probe	Genbank	Probability	Fold	Gene Symbol	Gene Name
33379_at	AB023140	0.00407	0.432	SSX2IP	synovial sarcoma, X breakpoint 2 interacting protein
31599_f_at	U10691	0.00390	0.420	MAGEA3	melanoma antigen, family A, 3
32935_at	AL080157	0.00389	0.512	WDR21	WD repeat domain 21
33072_at	AF041245	0.00361	0.809	HCRTR2	hypocretin (orexin) receptor 2
36777_at	AJ001687	0.00357	0.525	KLRK1	killer cell lectin-like receptor subfamily K, member 1
36269_at	AB002364	0.00356	0.538	ADAMTS3	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 3
38095_i_at	M83664	0.00351	0.596	HLA-DPB1	major histocompatibility complex, class II, DP beta 1
36272_r_at	X62167	0.00319	0.335	PMP2	peripheral myelin protein 2
494_at	U31120	0.00307	0.610	IL13	interleukin 13
34698_at	M60165	0.00300	0.522	GNAO1	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O
39646_at	S60415	0.00291	0.414	CACNB2	calcium channel, voltage-dependent, beta 2 subunit
36049_at	W27899	0.00278	0.497	---	CDNA clone IMAGE:4940887, partial cds
37039_at	J00194	0.00277	0.602	HLA-DRA	major histocompatibility complex, class II, DR alpha
37588_s_at	U62317	0.00262	0.621	MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2
33846_at	AA620377	0.00260	0.522	---	Cluster Incl. AA620377:ae57a07.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-950964
36416_g_at	AI688589	0.00259	0.512	CASK	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
1298_at	X86816	0.00256	0.447	---	Human estrogen receptor mRNA, alternatively spliced transcript H, partial cds.
40646_at	U27699	0.00235	0.562	CX3CR1	chemokine (C-X3-C motif) receptor 1
37108_at	X72755	0.00229	0.529	---	MRNA; cDNA DKFZp779B1535 (from clone DKFZp779B1535)
32997_at	AI018523	0.00228	0.363	GAGEB1	G antigen, family B, 1 (prostate associated)
35028_at	AB002314	0.00227	0.438	GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1
40679_at	AB004066	0.00213	0.458	SLC6A12	solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12
39498_at	AA044910	0.00213	0.497	---	Cluster Incl. X86400:H.sapiens mRNA for gamma subunit of sodium potassium ATPase
38833_at	U31767	0.00199	0.670	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1
35031_r_at	AF030514	0.00183	0.281	KIAA0316	KIAA0316 gene product
36911_at	M20681	0.00180	0.433	TYRP1	tyrosinase-related protein 1
31494_at	L12691	0.00175	0.434	---	Cluster Incl. D25272:Homo sapiens mRNA, clone-RES4-16
37782_at	AB000381	0.00170	0.654	SST	somatostatin
36767_at	X51420	0.00164	0.302	CYP1A1	cytochrome P450, family 1,

Affy Probe	Genbank	Probability	Fold	Gene Symbol	Gene Name
					subfamily A, polypeptide 1
35539_at	AB019246	0.00159	0.386	IMPG1	interphotoreceptor matrix proteoglycan 1
38330_at	X00457	0.00159	0.371	FRDA	Friedreich ataxia
35061_at	AF047492	0.00152	0.272	CXCL11	chemokine (C-X-C motif) ligand 11
34002_at	AL080151	0.00139	0.627	HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2
32017_at	U38805	0.00139	0.531	PARD6B	par-6 partitioning defective 6 homolog beta (C. elegans)
31398_at	D25272	0.00132	0.440	ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11
32451_at	X96744	0.00131	0.556	MS4A3	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)
34045_at	AF043469	0.00131	0.503	LOC196993	hypothetical protein LOC196993
36428_at	K03191	0.00130	0.569	VMD2	vitelliform macular dystrophy (Best disease, bestrophin)
AFFX-DapX-3_a	M33197	0.00122	0.469	---	L38424 B subtilis dapB, jojF, jojG genes corresponding to nucleotides 1358-3197 of L38424
31324_at	AF016492	0.00116	0.484	---	U82303:Homo sapiens unknown protein mRNA
32474_at	X85106	0.00111	0.644	PAX7	paired box gene 7
37219_at	AI636761	0.00098	0.395	CXCL9	chemokine (C-X-C motif) ligand 9
31506_s_at	AL080207	0.00097	0.288	DEFA1	defensin, alpha 1, myeloid-related sequence
378_s_at	W28432	0.00075	0.529	GML	GPI anchored molecule like protein
41820_s_at	D85376	0.00073	0.570	CDC2L5	cell division cycle 2-like 5 (cholinesterase-related cell division controller)
31310_at	U82303	0.00061	0.523	GLRA1	glycine receptor, alpha 1 (startle disease/hyperekplexia, stiff man syndrome)
39502_at	J03634	0.00046	0.553	DPYSL4	dihydropyrimidinase-like 4
35024_at	X14767	0.00031	0.272	OPRK1	opioid receptor, kappa 1
36220_at	Y12642	0.00030	0.346	DDAH1	dimethylarginine dimethylaminohydrolase 1
204_at	M13981	0.00022	0.601	HOXA4	homeo box A4
750_at	L38424	0.00021	0.389	TRHR	thyrotropin-releasing hormone receptor
33478_at	AA584202	0.00009	0.296	TNP2	transition protein 2 (during histone to protamine replacement)
1412_g_at	D84361	0.00008	0.560	CYP11B1	cytochrome P450, family 11, subfamily B, polypeptide 1

*Genes selected with a 1.0% false positive error rate for a total of 127 gene, 65 of these upregulated. Genes with no Locuslink classification are labeled with Genbank accession numbers

A hierarchical clustering of the Affymetrix patient data was performed using Cluster and Treeview, (by Michael Eisen, Stanford University) (Figure 3). The samples labeled as P are preeclamptic patients and the samples labeled as N are normal pregnant patients. The dataset was filtered from 12625 to 3564 genes using presence and expression criteria, and the resulting set was median-centered and normalized for genes and arrays. We used hierarchical clustering to analyze possible classes in genes. The above cluster includes sFlt1 along with other genes confirmed in literature.

From the predictive gene set, we found that expression of the gene for the following secreted polypeptides was upregulated in blood samples taken from women with pre-eclampsia: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein (#AL039458), leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, and galectin-3. We have also discovered that expression levels of the gene for the following secreted polypeptides were decreased in blood samples taken from women with pre-eclampsia: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, and azurocidin. In addition we also found the following intracellular polypeptides or enzymes that are increased in preeclamptic placenta sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2 and beta glucosidase. The following intracellular gene products/enzymes are decreased in preeclamptic placentas are: lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced

transcript H , chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11.

Example 2. mRNA expression of Flt-1 and sFlt-1 in pre-eclampsia.

As the above cluster identified sFlt1 along with other genes confirmed in the literature, we chose to confirm the ability of the array to identify predictive markers of pre-eclampsia using sFlt-1. For these experiments, mRNA expression of placental sFlt-1 from 3 patients with pre-eclampsia (P1, P2, P3) and three normotensive term pregnancies (N1, N2, N3) were determined by northern blot analysis (Figure 4). The higher band (7.5 kb) is the full length Flt-1 mRNA and the lower, more abundant band (3.4 kb) is the alternatively spliced sFlt-1 mRNA. Actin is included as a control and 28S is shown as arrowhead. These results show the increased expression of the gene for sFlt-1 in pre-eclamptic patients and confirm the use of the predictive gene set identified by the array as markers for pre-eclampsia or eclampsia or the propensity to develop pre-eclampsia or eclampsia.

Example 3. Immunohistochemistry analysis of Flt-1 expression in normal and pre-eclamptic patients.

In order to visualize Flt-1 expression in placental samples from normal and pre-eclamptic patients, a monoclonal antibody against human Flt-1 was used for immunohistochemistry analysis. Increased expression of Flt-1 by the syncitiotrophoblasts of the preeclamptic placenta was detected (Figure 5), further confirming the ability of the array to identify genes that can be used as markers for pre-eclampsia or eclampsia or the propensity to develop pre-eclampsia or eclampsia.

Other Embodiments

The description of the specific embodiments of the invention is presented for the purposes of illustration. It is not intended to be exhaustive or to limit the scope of the invention to the specific forms described herein. Although the invention has been described with reference to several embodiments, it will be understood by one of ordinary skill in the art that various modifications can be made without departing from the spirit and the scope of the invention, as set forth in the claims. All patents, patent applications, and publications referenced herein are hereby incorporated by reference. Other embodiments are in the claims.

What is claimed is:

Claims

1. A method of diagnosing a subject as having, or having a predisposition to, a pregnancy related hypertensive disorder, said method comprising measuring the level of at least one polypeptide, or a fragment thereof, in a sample from said subject, wherein said at least one polypeptide, or fragment thereof, is selected from the group consisting of follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase, and wherein an increase in the level of said at least one polypeptide, or fragment thereof, as compared to the level in a normal reference, is a diagnostic indicator of said pregnancy related hypertensive disorder or a predisposition to said pregnancy related hypertensive disorder.

2. The method of claim 1, wherein said increase is at least 20%.

3. The method of claim 1, wherein said polypeptide is selected from the group consisting of follistatin related protein, inhibin-A, beta fertilin, insulin-like growth factor binding protein-5, and secreted frizzled related protein.

4. A method of diagnosing a subject as having, or having a predisposition to, a pregnancy related hypertensive disorder, said method comprising measuring the level of at least one polypeptide, or a fragment thereof, in a sample from said subject, wherein said polypeptide, or fragment thereof, is selected from the group consisting of alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11, and wherein a decrease in the level of said polypeptide, or fragment thereof, as compared to a normal reference is a diagnostic indicator of said pregnancy related hypertensive disorder or a predisposition to said pregnancy related hypertensive disorder.

5. The method of claim 4, wherein said decrease is at least 20%.

6. The method of claim 1 or 4, wherein said pregnancy related hypertensive disorder is selected from the group consisting of pre-eclampsia, eclampsia, gestational hypertension, chronic hypertension, HELLP syndrome, and pregnancy with a small for gestational age (SGA) infant.

7. The method of claim 6, wherein said pregnancy related hypertensive disorder is pre-eclampsia or eclampsia.

8. The method of claim 1 or 4, wherein the normal reference is a sample previously taken from said subject.

9. The method of claim 1 or 4, further comprising measuring the level of at least one polypeptide, or fragment thereof, selected from the group consisting of soluble endoglin, sFlt-1, VEGF, and PlGF in a sample from said subject.

10. The method of claim 9, further comprising calculating the relationship between said levels of soluble endoglin, sFlt-1, VEGF, or PlGF using a metric, wherein an alteration in the relationship between said levels in the subject sample relative to said levels in a reference sample is a diagnostic indicator of a pregnancy related hypertensive disorder or a predisposition to a pregnancy related hypertensive disorder in said subject.

11. The method of claim 1 or 4, wherein said measuring is done using an immunological assay.

12. The method of claim 11, wherein said immunological assay is an ELISA.

13. The method of claim 1, wherein said method comprises measuring the level of at least two polypeptides or fragments thereof.

14. A method of diagnosing a subject as having, or having a predisposition to, a pregnancy related hypertensive disorder, said method comprising measuring the level of a nucleic acid molecule in a sample from said subject, said nucleic acid molecule selected from the group consisting of nucleic acids encoding follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine

receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase, wherein an increase in the level of said nucleic acid molecule, as compared to the level in a normal reference, is a diagnostic indicator of said pregnancy related hypertensive disorder or a predisposition to said pregnancy related hypertensive disorder.

15. A method of diagnosing a subject as having, or having a predisposition to, a pregnancy related hypertensive disorder, said method comprising measuring the level of a nucleic acid molecule in a sample from said subject, said nucleic acid molecule selected from the group consisting of nucleic acids encoding alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11, wherein a decrease in the level of said nucleic acid molecule, as compared to the level in a normal reference is a diagnostic indicator of said pregnancy related hypertensive disorder or a predisposition to said pregnancy related hypertensive disorder.

16. The method of claim 14 or 15, wherein the normal reference is a sample previously taken from said subject.

17. The method of claim 14 or 15, wherein said pregnancy related hypertensive disorder is selected from the group consisting of pre-eclampsia, eclampsia, gestational hypertension, chronic hypertension, HELLP syndrome, and pregnancy with a small for gestational age (SGA) infant.

18. The method of claim 17, wherein said pregnancy related hypertensive disorder is pre-eclampsia or eclampsia.

19. The method of claim 14 or 15, further comprising measuring the level of a nucleic acid molecule encoding a polypeptide selected from the group consisting of soluble endoglin, sFlt-1, VEGF, or PlGF in a sample from said subject.

20. The method of claim 1, 4, 14, or 15, wherein said subject is a non-pregnant human, a pregnant human, a post-partum human, or a non-human.

21. The method of claim 20, wherein said non-human is selected from the group consisting of a cow, a horse, a sheep, a pig, a goat, a dog, or a cat.

22. The method of claim 1, 4, 14, or 15, wherein said method is used to diagnose a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder, at least 4 weeks prior to the onset of symptoms.

23. The method of claim 1, 4, 14, or 15, wherein said sample is a bodily fluid, a tissue, or a cell in which said polypeptide or nucleic acid molecule is normally detectable.

24. The method of claim 23, wherein said bodily fluid is selected from the group consisting of blood, urine, amniotic fluid, saliva, serum, plasma, and cerebrospinal fluid.

25. A method of diagnosing a subject as having, or having a predisposition to, a pregnancy related hypertensive disorder, said method comprising determining the nucleic acid sequence of a gene encoding a polypeptide selected from the group consisting of follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, and beta glucosidase, wherein an alteration in the subject's nucleic acid sequence that is an alteration that increases the expression level or biological activity of the gene product in said subject diagnoses the subject with said pregnancy related hypertensive disorder or a predisposition to said pregnancy related hypertensive disorder.

26. A method of diagnosing a subject as having, or having a predisposition to a pregnancy related hypertensive disorder, said method comprising determining the nucleic acid sequence of a gene encoding a polypeptide selected from the group consisting of: alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-

alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11, wherein an alteration in the subject's nucleic acid sequence that is an alteration that decreases the expression level or biological activity of the gene product in said subject diagnoses the subject with said pregnancy related hypertensive disorder or a predisposition to said pregnancy related hypertensive disorder.

27. The method of claim 25 or 26, wherein said pregnancy related hypertensive disorder is selected from the group consisting of pre-eclampsia, eclampsia, gestational hypertension, chronic hypertension, HELLP syndrome, and pregnancy with a small for gestational age (SGA) infant.

28. The method of claim 27, wherein said pregnancy related hypertensive disorder is pre-eclampsia or eclampsia.

29. A kit for the diagnosis of a pregnancy related hypertensive disorder, or a predisposition to a pregnancy related hypertensive disorder in a subject comprising the following:

(a) a nucleic acid sequence or a sequence complementary thereto selected from the group consisting of nucleic acids sequences that encode any of the following polypeptides: follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral

endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11; and

(b) instructions for the use of said nucleic acid sequence or sequence complementary thereto for the diagnosis of a pregnancy related hypertensive disorder in a subject.

30. The kit of claim 29, further comprising a reference sample, standard, or level.

31. The kit of claim 30, wherein said reference sample is sample from a subject not having a pregnancy related disorder or a subject that is not pregnant.

32. A kit for the diagnosis of a pregnancy related hypertensive disorder in a subject comprising the following:

(a) a component useful for detecting a polypeptide selected from the group consisting of follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral

endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11; and

(b) instructions for the use of said component for the diagnosis of a pregnancy related hypertensive disorder in said subject.

33. The kit of claim 32, wherein said component is a binding molecule that specifically binds said polypeptide.

34. The kit of claim 33, wherein said binding molecule is an antibody, or antigen-binding fragment thereof, that specifically binds said polypeptide.

35. The kit of claim 32, further comprising a reference sample, standard, or level.

36. The kit of claim 35, wherein said reference sample is a sample from a subject not having a pregnancy related disorder or a subject that is not pregnant.

37. The kit of claim 32, wherein said polypeptide is detected by an assay selected from the group consisting of an immunological assay, an enzymatic assay, and a colorimetric assay.

38. A nucleic acid array comprising one or more substrate supports that are stably associated with a plurality of polynucleotide probes, wherein said polynucleotide probes are capable of hybridizing under highly stringent conditions to RNA transcripts, or the complements thereof, of genes encoding proteins selected from the group consisting of follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11.

39. A polypeptide array comprising one or more substrate supports that are stably associated with a plurality of polypeptides selected from the group consisting of follistatin related protein, interleukin 8, inhibin A, VEGF-C, angiogenin, beta fertilin, hypothetical protein, leukocyte associated Ig-like receptor secreted protein, erythroid differentiation protein, adipogenesis inhibitory factor, corticotropin releasing factor binding protein, alpha-1 anti-chymotrypsin, insulin-like growth factor binding protein-5, CD33L, cytokine receptor like factor 1, platelet derived endothelial growth factor, lysyl hydroxylase isoform 2, stanniocalcin precursor, secreted frizzled related

protein, galectin-3, sperminine oxidase, UDP glycosyltransferase 2 family polypeptide B28, neurotrophic tyrosine kinase receptor 2, neutral endopeptidase, CDC28 protein kinase regulatory subunit 2, beta glucosidase, alpha defensin, ADAM-TS3, cholecystokinin precursor, interferon stimulated T-cell alpha chemoattractant precursor, azurocidin, lanosterol synthase, calcium/calmodulin-dependent serine protein kinase, estrogen receptor-alternatively spliced transcript H, chemokine (CX3C motif) receptor 1, tyrosinase-related protein 1, hydoxy-delta-5-steroid dehydrogenase, dihydropyramidinase-like-4, and cytochrome P450-family 11; variants of said polypeptides; antibodies specific for said polypeptides or variants; or any combination of said polypeptides, variants, or antibodies.

40. The array of claim 38 or 39, further comprising instructions for the use of said arary for the diagnosis of a pregnancy related hypertensive disorder in said subject.

Figure 1

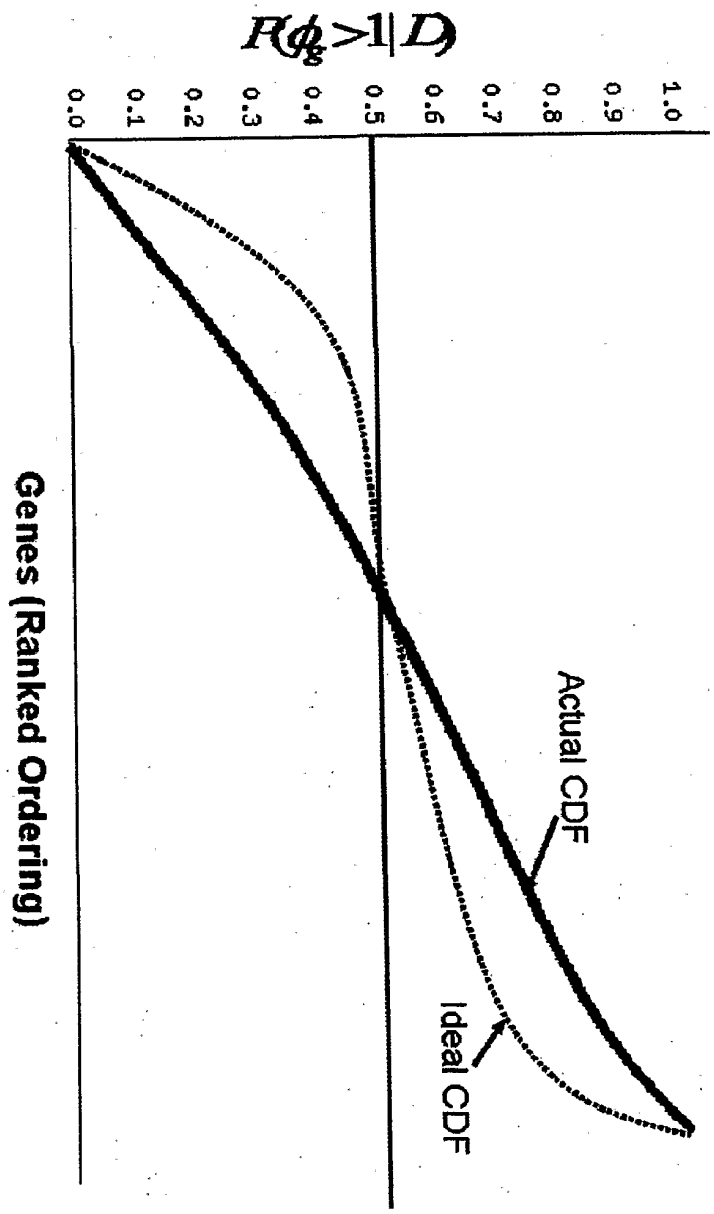
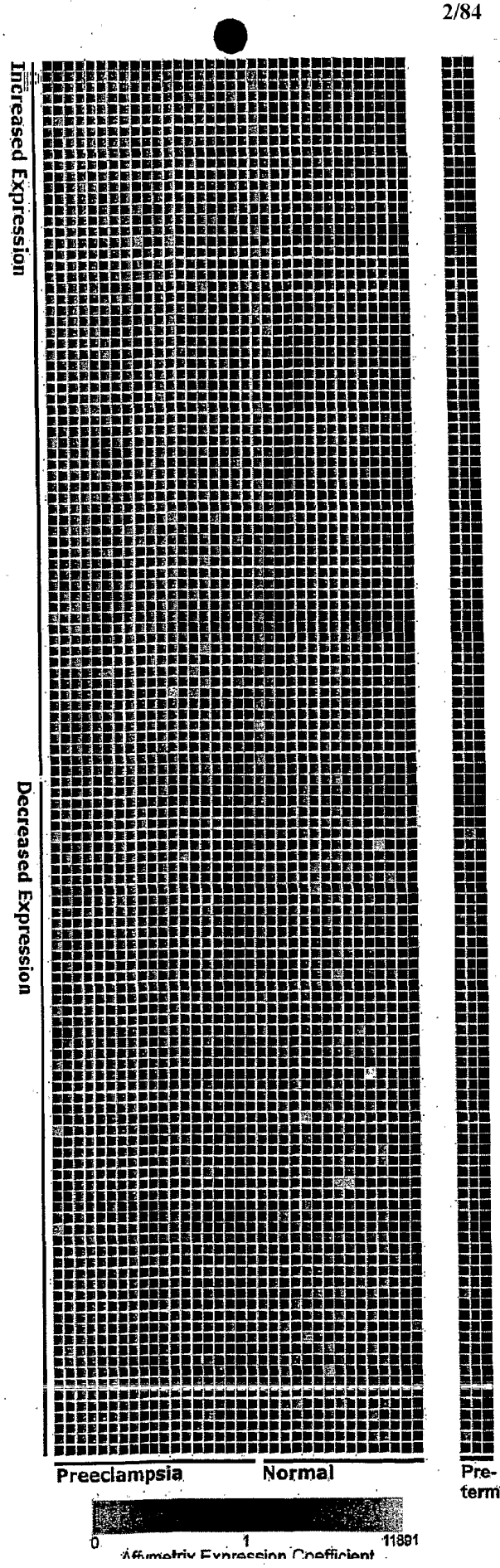

$F(\phi_g > 1 | D)$
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Genes (Ranked Ordering)
Actual CDF
Ideal CDF

Figure 2



U76702	follistatin-like 3 (secreted glycoprotein)
X51602	fms-related tyrosine kinase 1 (VEGF/vascular permeability factor receptor)
X51602	fms-related tyrosine kinase 1 (VEGF/vascular permeability factor receptor)
M11567	insulin-like growth factor binding protein 5
U07057	coronin, actin binding protein, 2A
J03779	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CD10)
U37143	cytochrome P450, family 2, subfamily J, polypeptide 2
Y16270	periaxinrin
L27559	glyceraldehyde-3-phosphate dehydrogenase
U61836	vascular endothelial growth factor C
AI650015	ATP-binding cassette, sub-family A (ABC1), member 12
D16154	Transcription Factor Eb
U43142	fms-related tyrosine kinase 1 (VEGF/vascular permeability factor receptor)
D86356	syntaxin binding protein 5-like
H04740	angiogenin, ribonuclease, RNase A family, 5
X52009	inhibin, alpha
U01134	spermine oxidase
M74297	fms-related tyrosine kinase 1 (VEGF/vascular permeability factor receptor)
L35849	a disintegrin and metalloproteinase domain 2 (fertilin beta)
M77144	guanylate cyclase activator 2A (guanylin)
AF056089	ribosomal protein S6 kinase, 90kDa, polypeptide 2
W27723	protein phosphatase 1, regulatory (inhibitor) subunit 16B
X53004	basic helix-loop-helix domain containing, class B, 2
AF055033	glycophorin E
AF052095	nebulette
L37362	leucine-rich repeats and immunoglobulin-like domains 1
L26953	solute carrier family 2 (facilitated glucose transporter), member 3
AF091582	UDP glycosyltransferase 2 family, polypeptide B28
U20350	inhibin, beta A (activin A, activin AB alpha polypeptide)
U01134	fms-related tyrosine kinase 1 (VEGF/vascular permeability factor receptor)
AB001915	nuclear receptor subfamily 5, group A, member 2
X86400	neurotrin
X68723	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
X03789	neurotrophic tyrosine kinase, receptor, type 2
U12595	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
AL022458	sialic acid binding Ig-like lectin 6
S77812	src homology 2 domain containing transforming protein C3
U43733	neurotrophic tyrosine kinase, receptor, type 2
AB020630	insulin-like growth factor binding protein 5
AB023223	neurexophilin 4
Y12673	lymphocyte antigen 6 complex, locus D
M97496	serine (or cysteine) proteinase inhibitor, clade A
D83402	prostaglandin I2 (prostacyclin) synthase
AL079273	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51
W26403	TNF receptor-associated factor 3 interacting protein 1
Z29083	trophoblast glycoprotein
U37143	cytochrome P450, family 2, subfamily J, polypeptide 2
AF042165	filamin B, beta (actin binding protein 278)
X55022	corticotropin releasing hormone binding protein
M90356	Human transforming growth factor beta-3 gene, 5 end
M25753	cyclin B1
AF009624	kinesin family member 17
D87953	N-myc downstream regulated gene 1
X72012	endoglin (Osler-Rendu-Weber syndrome 1)
N66619	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, s
M28130	interleukin 6
M65062	insulin-like growth factor binding protein 5
AF066904	CHK2 checkpoint homolog (S. pombe)
X54942	CDC28 protein kinase regulatory subunit 2
U52111	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
M30704	amphiregulin (schwannoma-derived growth factor)
D86976	minor histocompatibility antigen HA-1
X64824	POU domain, class 4, transcription factor 1
J03060	glucosidase, beta, acid, pseudogene
AF043458	prostate stem cell antigen
X60382	collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)
AB006313	paired box gene 4
AB018311	latrophilin 3
U83233	nuclear receptor subfamily 1, group H, member 4
X65879	empty spiracles homolog 1 (Drosophila)
U22526	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
M76482	desmoglein 3 (pemphigus vulgaris antigen)
U40462	zinc finger protein, subfamily 1A, 1 (Ikaros)
U10689	melanoma antigen, family A, 6
AB028140	synovial sarcoma, X breakpoint 2 interacting protein
U10691	melanoma antigen, family A, 3
AL030157	VID repeat domain 21
AF041245	hypocretin (orexin) receptor 2
AJ001687	killer cell lectin-like receptor subfamily K, member 1
AB002364	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1
M83664	major histocompatibility complex, class II, DP beta 1
X62157	peripheral myelin protein 2
U51120	interleukin 13
M60165	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O
S60415	calcium channel, voltage-dependent, beta 2 subunit
W27999	CDNA clone IMAGE:49D687, partial cds
J00194	major histocompatibility complex, class II, DR alpha
U02317	mitogen-activated protein kinase 9 interacting protein 2
AA620377	Cluster Incl. AA620377:se57e07.s1 Homo sapiens cDNA, 3 end
AI688589	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
X66616	Human estrogen receptor mRNA, alternatively spliced transcript H, partial cds
U27699	chemokine (C-X3-C motif) receptor 1
X72755	MRNA; cDNA DKFZp779B1535 (from clone DKFZp779B1535)
AI018523	G antigen, family E, 1 (prostate associated)
AB002314	gamma-aminobutyric acid (GABA) A receptor, beta 1
AB004066	solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12
AA844910	X86400 H.sapiens mRNA for gamma subunit of sodium potassium ATPase
U31767	major histocompatibility complex, class II, DP alpha 1
AF030514	KIAA0316 gene product
M20581	tyrosinase-related protein 1
L12691	Cluster Incl. D25272:Homo sapiens mRNA, clone-RES4-16
AB000381	sequestrin
X51429	cytochrome P450, family 1, subfamily A, polypeptide 1
AB019245	interphotoreceptor matrix proteoglycan 1
X00457	Friedreich ataxia
AF047462	chemokine (C-X-C motif) ligand 11
AL050151	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2
U19905	par-6 partitioning defective 6 homolog beta (C. elegans)
D25272	ATP-binding cassette, sub-family B (MDR/TAP), member 11
X98744	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)
AF043469	hypothetical protein LOC196993
K03191	vitelliform macular dystrophy (Best disease, bestrophin)
M23197	L38424 B subtilis dapE, jojF, jojG genes corresponding to nucleotides 1358-3197
AF016492	U92333:Homo sapiens unknown protein mRNA,
X85106	paired box gene 7
AI535761	chemokine (C-X-C motif) ligand 9
AL080207	defensin, alpha 1, myeloid-related sequence
W26432	GPI anchored molecule like protein
D85376	cell division cycle 2-like 5 (cholinesterase-related cell division controller)
U82303	glycine receptor, alpha 1 (startle disease/hyperekplexia, Kok man syndrome)
J03824	dihydropyrimidinase-like 5
X14767	opioid receptor, kappa 1
Y12642	dimethylarginine dimethylaminohydrolase 1
M13981	homeo box A4
L38484	thyrotropin-releasing hormone receptor
AA584232	transition protein 2 (during histone to protamine replacement)
D64301	cytochrome P450, family 11, subfamily B, polypeptide 1

Figure 3

Figure 4

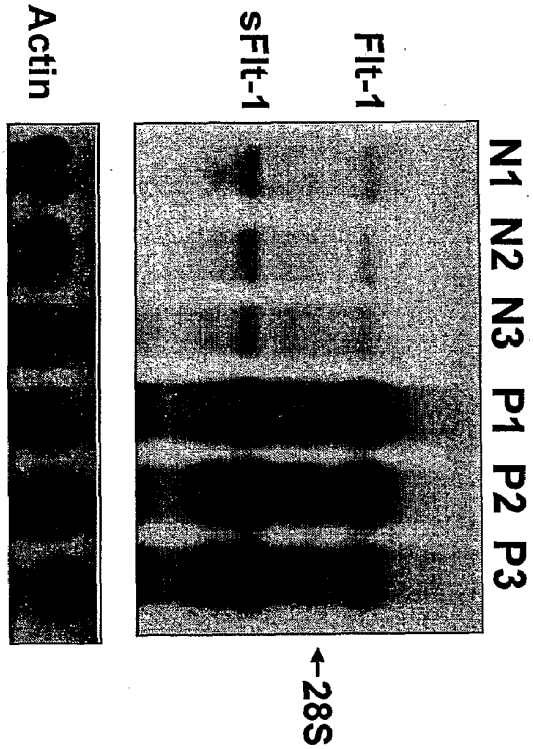
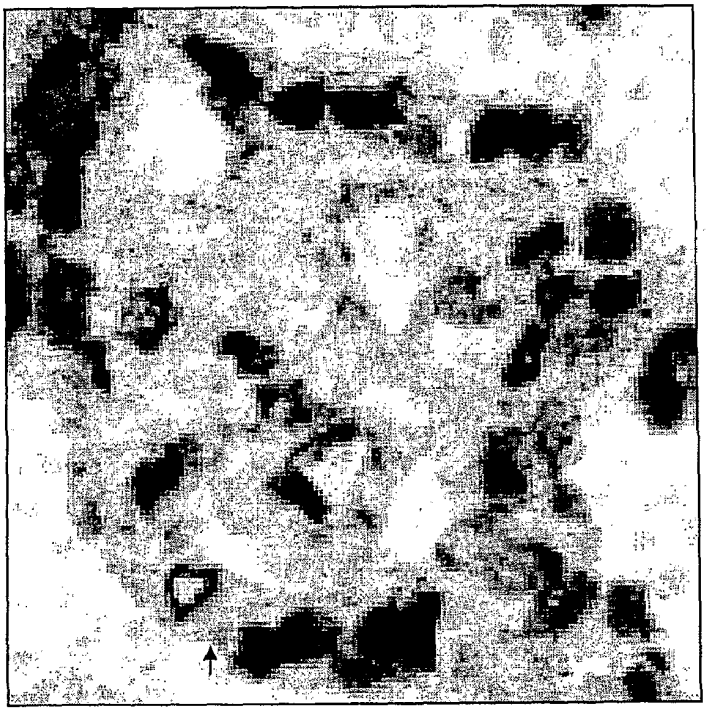
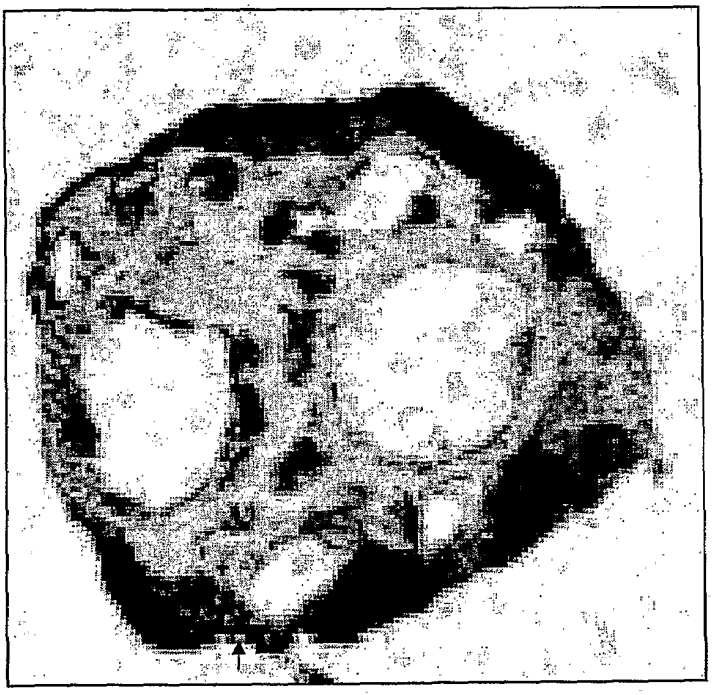


Figure 5



Normal placenta



Preeclamptic placenta

Figure 6A

MRPGAPGPLWPLPWGALAWAVGFVSSMGSGNPAPGGVCWLQQGQEATCSLVLQTDVTRAEC
CASGNIDTAWSNLTHPGNKINLLGFLGLVHCLPCKDSCDGVECGPGKACRMLGGRPRCECAPD
CSGLPARLQVCGSDGATYRDECELRAARCRGHPDLSVMYRGRCRKSCEHVVCPRPQSCVVDQ
TGSAHCVVCRAAPCPVPSSPGQELCGNNNVTYISSCHMRQATCFLGRSIGVRHAGSCAGTPEE
PPGGESAEEEENFV

Figure 6B

gttcgccatg cgtcccgggg cgccagggcc actctggcct ctgccctggg gggccctggc ttgggccgtg ggcttcgtga gctccatggg
ctcggggaac cccgcgcccg gtggtgtttg ctggctccag cagggccagg aggccacctg cagcctggtg ctccagactg atgtcacccg
ggccgagtgc tgtgcctccg gcaacattga caccgcctgg tccaacctca cccacccggg gaacaagatc aacctcctcg gcttcttggg
ccttgtccac tgccttccct gcaaagattc gtgcgacggc gtggagtgcg gcccgggcaa ggcgtgccgc atgctggggg gccgcccgcg
ctgcgagtgc gcgcccgact gctcggggct cccggcgcgg ctgcaggtct gcggctcaga cggcgccacc taccgcgacg agtgcgagct
gcgcgccgcg cgctgccgcg gccacccgga cctgagcgtc atgtaccggg gccgctgccg caagtcctgt gagcacgtgg tgtgcccgcg
gccacagtcg tgcgtcgtgg accagacggg cagcgcccac tgcgtggtgt gtcgagcggc gccctgccct gtgccctcca gccccggcca
ggagctttgc ggcaacaaca acgtcaccta catctcctcg tgccacatgc gccaggccac ctgcttcctg ggccgctcca tcggcgtgcg
ccacgcgggc agctgcgcag gcacccctga ggagccgcca ggtggtgagt ctgcagaaga ggaagagaac ttcgtgtgag cctgcaggac
aggcctgggc ctggtgcccg aggccccccа tcatcccctg ttatttattg ccacagcaga gtctaattta tatgccacgg acactcctta
gagcccggat tcggaccact tggggatccc agaacctccc tgacgatatc ctggaaggac tgaggaaggg aggcctgggg gccggctggt
gggtgggata gacctgcgtt ccggacactg agcgcctgat ttagggccct tctctaggat gccccagccc ctaccctaag acctattgcc
ggggaggatt ccacacttcc gctcctttgg ggataaacct attaattatt gctactatca agagggctgg gcattctctg ctggtaattc
ctgaagaggc atgactgctt ttctcagccc caagcctcta gtctgggtgt gtacggaggg tctagcctgg gtgtgtacgg agggtctagc
ctgggtgagt acggagggtc tagcctgggt gagtacggag ggtctagcct gggtgagtac ggagagtcta gcctgggtgt gtatggagga
tctagcctgg gtgagtatgg agggtctagc ctgggtgagt atggagggtc tagcctgggt gtgtatggag ggtctagcct gggtgagtat
ggagggtcta gcctgggtgt gtatggaggg tctagcctgg gtgagtatgg agggtctagc ctgggtgtgt acggagggtc tagtctgagt
gcgtgtgggg acctcagaac actgtgacct tagcccagca agccaggccc ttcatgaagg ccaagaaggc tgccaccatt ccctgccagc
ccaagaactc cagcttcccc actgcctctg tgtgcccctt tgcgtcctgt gaaggccatt gagaaatgcc cagtgtgccc cctgggaaag
ggcacggcct gtgctcctga cacgggctgt gcttggccac agaaccaccc agcgtctccc ctgctgctgt ccacgtcagt tcatgaggca
acgtcgcgtg gtctcagacg tggagcagcc agcggcagct cagagcaggg cactgtgtcc ggcggagcca agtccactct gggggagctc
tggcggggac cacgggccac tgctcaccca ctggccccga ggggggtgta gacgccaaga ctcacgcatg tgtgacatcc ggagtcctgg
agccgggtgt cccagtggca ccactaggtg cctgctgcct ccacagtggg gttcacaccc agggctcctt ggtcccccac aacctgcccc
ggccaggcct gcagacccag actccagcca gacctgcctc acccaccaat gcagccgggg ctggcgacac cagccaggtg ctggtcttgg
gccagttctc ccacgacggc tcaccctccc ctccatctgc gttgatgctc agaatcgcct acctgtgcct gcgtgtaaac cacagcctca
gaccagctat ggggagagga caacacggag gatatccagc ttccccggtc tggggtgagg agtgtgggga gcttgggcat cctcctccag
cctcctccag cccccaggca gtgccttacc tgtggtgccc agaaaagtgc ccctaggttg gtgggtctac aggagcctca gccaggcagc
ccaccccacc ctggggccct gcctcaccaa ggaaataaag actcaaagaa gccttttttt ttttttttt

Figure 7A

MTSKLAVALLAAFLISAALCEGAVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIV
KLSDGRELCLDPKENWVQRVVEKFLKRAENS

Figure 7B

gaattcagta acccaggcat tattttatcc tcaagtctta ggttggttgg agaaagataa caaaaagaaa catgattgtg cagaaacaga caaacctttt tggaaagcat ttgaaaatgg cattcccccc ccacagtgtg ttcacagtgt gggcaaattc actgctctgt cgtactttct gaaaatgaag aactgttaca ccaaggtgaa ttatttataa attatgtact tgcccagaag cgaacagact tttactatca taagaaccct tccttggtgt gctctttatc tacagaatcc aagacctttc aagaaaggtc ttggattctt ttcttcagga cactaggaca taaagccacc ttttatgat ttgttgaaat ttctcactcc atccctttg ctgatgatca tgggtcctca gaggtcagac ttggtgtcct tggataaaga gcatgaagca acagtggctg aaccagagtt ggaacccaga tgctctttcc actaagcata caactttcca ttagataaca cctccctccc accccaacca agcagctcca gtgcaccact ttctggagca taaacatacc ttaactttac aacttgagtg gccttgaata ctgttcctat ctggaatgtg ctgttctctt tcatcttcct ctattgaagc cctcctattc ctcaatgcct tgctccaact gcctttggaa gattctgctc ttatgcctcc actggaatta atgtcttagt accacttgtc tattctgcta tatagtcagt ccttacattg ctttcttctt ctgatagacc aaactcttta aggacaagta cctagtctta tctatttcta gatcccccac attactcaga aagttactcc ataaatgttt gtggaactga tttctatgtg aagacatgtg ccccttcact ctgttaacta gcattagaaa aacaaatctt ttgaaaagtt gtagtatgcc cctaagagca gtaacagttc ctagaaactc tctaaaatgc ttagaaaaag atttatttta aattacctcc ccaataaaat gattggctgg cttatcttca ccatcatgat agcatctgta attaactgaa aaaaaataat tatgccatta aaagaaaatc atccatgatc ttgttctaac acctgccact ctagtactat atctgtcaca tggtctatga taaagttatc tagaaataaa aaagcataca attgataatt caccaaattg tggagcttca gtattttaaa tgtatattaa aattaaatta ttttaaagat caaagaaaac tttcgtcata ctccgtattt gataaggaac aaataggaag tgtgatgact caggtttgcc ctgaggggat gggccatcag ttgcaaatcg tggaatttcc tctgacataa tgaaaagatg agggtgcata agttctctag tagggtgatg atataaaaag ccaccggagc actccataag gcacaaactt tcagagacag cagagcacac aagcttctag gacaagagcc aggaagaaac caccggaagg aaccattctc actgtgtgta aacatgactt ccaagctggc cgtggctctc ttggcagcct tcctgatttc tgcagctctg tgtgaaggta agcacatctt tctgacctac agcgttttcc tatgtctaaa tgtgatcctt agatagcaaa gctattcttg atgctttggt aacaaacatc ctttttattc agaaacagaa tataatctta gcagtcaatt aatgttaaat tgaagattta gaaaaaacta tatataacac ttaggaaata taaaggtttg atcaatatag atattctgct tttataattt ataccaggta gcatgcatat atttaacgta aataagtaat ttatagtatg tcctattgag aaccacggtt acctatatta tgtattaata ttgagttgag caaggtaact cagacaattc cactccttgt agtatttcat tgacaagcct cagatttgtc attaattcct gtctggttta aagataccct gattatagac caggcatgta taacttattt atatatttct gttaattctt tctgaaggca atttctatgc tggagagtct tagcttgcct actataaata acactgtggt atcacagagg attatgcaat attgaccaga taaaaatacc atgaagatgt tgatattgta caaaaagaac tctaactctt atataggaag ttgttcaatg ttgtcagtta tgactgtttt ttaaaacaaa gaactaactg aggtcaaggg ctaggagata ttcaggaatg agttcactag aaacatgatg ccttccatag tctccaaata atcatattgg aattagaagg aagtagctgg cagagctgtg cctgttgata aaatcaatcc ttaatcactt tttcccccaa caggtgcagt tttgccaagg agtgctaaag aacttagatg tcagtgcata aagacatact ccaaaccttt ccaccccaaa tttatcaaag aactgagagt gattgagagt ggaccacact gcgccaacac agaaattatg taagtacttt aaaaaagatt agatattttg ttttagcaaa cttaaaatta aggaaggtgg aaatatttag gaaagttcca ggtgttagga ttacagtagt aaatgaaaca aaacaaaata aaaatatttg tctacatgac atttaaatat ggtagcttcc acaactacta taaatgttat tttggactta gactttatgc ctgacttaag gaatcatgat ttgaatgcaa aaactaaata ttaatctgaa ccatttcttt cttatttcag tgtaaagctt tctgatggaa gagagctctg tctggacccc aaggaaaact gggtgcagag ggttgtggag aagttttga agaggtaagt tatatatttt ttaatttaaa tttttcattt atcctgagac atataatcca aagtcagcct ataaatttct ttctgttgct aaaaatcgtc attaggtatc tgccttttg gttaaaaaaa aaggaatagc atcaatagtg agtttgttgt acttatgacc agaaagacca tacatagttt gcccaggaaa ttctgggttt aagcttgtgt cctatactct tagtaaagtt ctttgtcact cccagtagtg tcctatttta gatgataatt tctttgatct ccctatttat agttgagaat atagagcatt tctaacacat gaatgtcaaa gactatattg acttttcaag aacctactt tccttcttat taaacatagc tcatctttat attttaatt ttattttagg gctgagaatt cataaaaaaa ttcattctct gtggtatcca agaatcagtg aagatgccag tgaaacttca agcaaatcta cttcaacact tcatgtattg tgtgggtctg ttgtagggtt gccagatgca atacaagatt cctggttaaa tttgaatttc agtaaacaat gaatagtttt tcattgtacc atgaaatatc cagaacatac ttatatgtaa agtattattt atttgaatct

Figure 7B (Continued)

acaaaaaaca acaaataatt tttaaatata aggattttcc tagatattgc acgggagaat atacaaatag caaaattggg ccaagggcca agagaatatc cgaactttaa tttcaggaat tgaatgggtt tgctagaatg tgatatttga agcatcacat aaaaatgatg ggacaataaa ttttgccata aagtcaaatt tagctggaaa tcctggattt ttttctgtta aatctggcaa ccctagtctg ctagccagga tccacaagtc cttgttccac tgtgccttgg tttctccttt atttctaagt ggaaaaagta ttagccacca tcttacctca cagtgatgtt gtgaggacat gtggaagcac tttaagtttt ttcatcataa cataaattat tttcaagtgt aacttattaa cctatttatt atttatgtat ttatttaagc atcaaatatt tgtgcaagaa tttggaaaaa tagaagatga atcattgatt gaatagttat aaagatgtta tagtaaattt attttatttt agatattaaa tgatgtttta ttagataaat ttcaatcagg gtttttagat taaacaaaca aacaattggg tacccagtta aattttcatt tcagatatac aacaaataat tttttagtat aagtacatta ttgtttatct gaaattttaa ttgaactaac aatcctagtt tgatactccc agtcttgtca ttgccagctg tgttggtagt gctgtgttga attacggaat aatgagttag aactattaaa acagccaaaa ctccacagtc aatattagta atttcttgct ggttgaaact tgtttattat gtacaaatag attcttataa tattatttaa atgactgcat ttttaaatac aaggctttat atttttaact ttagtgtttt tatgtgctct ccaaattttt tttactgttt ctgattgtat ggaaatataa aagtaaatat gaaacattta aaatataatt tgttgtcaaa gtaatcaagt gtttgtcttt tttttagttt tagcttattg ggattctctt tgtttatatt taaaattata ctttgattta gaaaacataa atgcttcccc ttagcatttt gttatggaaa attacaaact tttatttta gaaaacagaa ctcctttcca gaaataggtt acaaacagta gtgtcctcca cagaatgttg gaaatgtttt caactcccca ctgtatacta tcttgctaat aagtctgtct tcagatttcg attaaccggt ttgtatgtct gtgcacttta gcatagctgg acattaaaga ggaaagagag tacatattat aagttgctta tcagtaactg aggagtaaaa ctgataaatg tgaggcaaag aagtttaaaa tatggttaaa gcctaagcat atttgcaaac aaatcaaaca atactctgag aagtaaaaac ataattattt aattaacaaa tttcagtgga taaattttat aacaaattag acacagttga aaataaaatt agaaaactag aaaatagaac aaaagaaact tctggaattc a

Figure 8A

MVLHLLLFLLLTPQGGHSCQGLELARELVLAKVRALFLDALGPPAVTREGGDPGVRRLPRRHAL
GGFTHRGSEPEEEEDVSQAILFPATDASCEDKSAARGLAQEAEEGLFRYMFRPSQHTRSRQVT
SAQLWFHTGLDRQGTAASNSSEPLLGLLALSPGGPVAVPMSLGHAPPHWAVLHLATSALSLLTH
PVLVLLLRCPLCTCSARPEATPFLVAHTRTRPPSGGERARRSTPLMSWPWSPSALRLLQRPPEE
PAAHANCHRVALNISFQELGWERWIVYPPSFIFHYCHGGCGLHIPPNLSLPVPGAPPTPAQPYSL
LPGAQPCCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQHCACI

Figure 8B

gaaggactgg ggaagactgg atgagaaggg tagaagaggg tgggtgtggg atggggaggg gagagtggaa aggccctggg
cagaccctgg cagaaggggc acggggcagg gtgtgagttc cccactagca gggccaggtg agctatggtg ctgcacctac tgctcttctt
gctgctgacc ccacagggtg ggcacagctg ccaggggctg gagctggccc gggaacttgt tctggccaag gtgagggccc tgttcttgga
tgccttgggg cccccgcgg tgaccaggga aggtggggac cctggagtca ggcggctgcc ccgaagacat gccctggggg gcttcacaca
caggggctct gagcccgagg aagaggagga tgtctcccaa gccatccttt tcccagccac agatgccagc tgtgaggaca agtcagctgc
cagagggctg gcccaggagg ctgaggaggg cctcttcaga tacatgttcc ggccatccca gcatacacgc agccgccagg tgacttcagc
ccagctgtgg ttccacaccg ggctggacag gcagggcaca gcagcctcca atagctctga gcccctgcta ggcctgctgg cactgtcacc
gggaggaccc gtggctgtgc ccatgtcttt gggccatgct ccccctcact gggccgtgct gcacctggcc acctctgctc tctctctgct
gacccacccc gtcctggtgc tgctgctgcg ctgtcccctc tgtacctgct cagcccggcc tgaggccacg cccttcctgg tggcccacac
tcggaccaga ccacccagtg gaggggagag agcccgacgc tcaactcccc tgatgtcctg gccttggtct ccctctgctc tgcgcctgct
gcagaggcct ccggaggaac cggctgccca tgccaactgc cacagagtag cactgaacat ctccttccag gagctgggct gggaacggtg
gatcgtgtac cctcccagtt tcatcttcca ctactgtcat ggtggttgtg ggctgcacat cccaccaaac ctgtcccttc cagtccctgg
ggctcccct accccagccc agccctactc cttgctgcca ggggcccagc cctgctgtgc tgctctccca gggaccatga ggcccctaca
tgtccgcacc acctcggatg gaggttactc tttcaagtat gagacagtgc ccaaccttct cacgcagcac tgtgcttgta tctaagggtg
gggggtcttc cttcttaatc ccatggctgg tggccacgcc cccaccatca tcagctggga ggaaaggcag agttgggaaa tagatggc

Figure 9A

MHLLGFFSVACSLLAAALLPGPREAPAAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQLRSVSS
VDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILKSIDNEWRKT
QCMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSYLSKTLFEITVPLS
QGPKPVTISFANHTSCRCMSKLDVYRQVHSIIRRSLPATLPQCQAANKTCPTNYMWNNHICRCL
AQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLRPASCGPHKELDRNSCQCVC
KNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCACECTESPQKCLLKGKKFHHQTC
SCYRRPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS

Figure 9B

cgcggggtgt tctggtgtcc cccgccccgc ctctccaaaa agctacaccg acgcggaccg cggcggcgtc ctccctcgcc ctcgcttcac
ctcgcgggct ccgaatgcgg ggagctcgga tgtccggttt cctgtgaggc ttttacctga cacccgccgc ctttccccgg cactggctgg
gagggcgccc tgcaaagttg ggaacgcgga gccccggacc cgctcccgcc gcctccggct cgcccagggg gggtcgccgg
gaggagcccg ggggagaggg accaggaggg gcccgcggcc tcgcaggggc gcccgcgccc ccacccctgc ccccgccagc
ggaccggtcc cccacccccg gtccttccac catgcacttg ctgggcttct tctctgtggc gtgttctctg ctcgccgctg cgctgctccc
gggtcctcgc gaggcgcccg ccgccgccgc cgccttcgag tccggactcg acctctcgga cgcggagccc gacgcgggcg aggccacggc
ttatgcaagc aaagatctgg aggagcagtt acggtctgtg tccagtgtag atgaactcat gactgtactc tacccagaat attggaaaat
gtacaagtgt cagctaagga aaggaggctg gcaacataac agagaacagg ccaacctcaa ctcaaggaca gaagagacta taaaatttgc
tgcagcacat tataatacag agatcttgaa aagtattgat aatgagtgga gaaagactca atgcatgcca cgggaggtgt gtatagatgt
ggggaaggag tttggagtcg cgacaaacac cttctttaaa cctccatgtg tgtccgtcta cagatgtggg ggttgctgca atagtgaggg
gctgcagtgc atgaacacca gcacgagcta cctcagcaag acgttatttg aaattacagt gcctctctct caaggcccca aaccagtaac
aatcagtttt gccaatcaca cttcctgccg atgcatgtct aaactggatg tttacagaca agttcattcc attattagac gttccctgcc
agcaacacta ccacagtgtc aggcagcgaa caagacctgc cccaccaatt acatgtggaa taatcacatc tgcagatgcc tggctcagga
agattttatg ttttcctcgg atgctggaga tgactcaaca gatggattcc atgacatctg tggaccaaac aaggagctgg atgaagagac
ctgtcagtgt gtctgcagag cggggcttcg gcctgccagc tgtggacccc acaaagaact agacagaaac tcatgccagt gtgtctgtaa
aaacaaactc ttccccagcc aatgtggggc caaccgagaa tttgatgaaa acacatgcca gtgtgtatgt aaaagaacct gccccagaaa
tcaaccccta aatcctggaa aatgtgcctg tgaatgtaca gaaagtccac agaaatgctt gttaaaagga aagaagttcc accaccaaac
atgcagctgt tacagacggc catgtacgaa ccgccagaag gcttgtgagc caggattttc atatagtgaa gaagtgtgtc gttgtgtccc
ttcatattgg aaaagaccac aaatgagcta agattgtact gttttccagt tcatcgattt tctattatgg aaaactgtgt tgccacagta gaactgtctg
tgaacagaga gacccttgtg ggtccatgct aacaaagaca aaagtctgtc tttcctgaac catgtggata actttacaga aatggactgg
agctcatctg caaaaggcct cttgtaaaga ctggttttct gccaatgacc aaacagccaa gattttcctc ttgtgatttc tttaaaagaa
tgactatata atttatttcc actaaaaata ttgtttctgc attcattttt atagcaacaa caattggtaa aactcactgt gatcaatatt tttatatcat
gcaaaatatg tttaaaataa aatgaaaatt gtatt

Figure 10A

MVMGLGVLLLVFVLGLGLTPPTLAQDNSRYTHFLTQHYDAKPQGRDDRYCESIMRRRGLTSPCK
DINTFIHGNKRSIKAICENKNGNPHRENLRISKSSFQVTTCKLHGGSPWPPCQYRATAGFRNVVV
ACENGLPVHLDQSIFRRP

Figure 10B

tgtttgcatt aagttcatag attataattt gtaatggaat caacaccaaa tgcaaattag aaagagagcc cactttgctc acccagtcac
gtcttcccat gtaaccatag aacgttgggg tcctgtgtct ttctagatcc acagtcttgc tctcagaaca ggctagccac accacaggcc
tagtgccagg acccatggcc ttttttaag ctcagactcc cttctgtgaa cagcaatatc cccacaactt gtacaacatt ggtgcttcct
gcaagggcta cagaactatt tgatacgaaa atgttcattg acttacacac aagagaagca caaaataaaa aattaataat taatttaatg
tctttgaaaa tgtaccattt atttttacat ttggggtcat aagaattgta ttacacttaa gaatgcaata caatttgaag atcagatttt tctccctttg
tgagaatttc tcagtatgtg tgatgactac caagaaatca tagccagtca taaattcagt gagttactca taaacgaaca agaaccacct
acttcttggg gaggtaggtc tgcttccctt caactcagga tacaactgct ttcaactgct ttcttcacat tagctgacta attagctaga agcctgtcgt
aaacaatttt atggttgact ccttccctgg gctcagggtt ccctagaaca gagaggtccc caaatcccgg tctgtggcct gtccgcctaa
gctctgcctc ctgccagatc agcaggcagc attagattct cataggagct ggacgcctat tgtgaactgc gcatgtgcgg gatccagatt
gtgcactctt tatgagaatc taactaatgc ttgatgatct atctgaacca gaacaatttc atcctgaaac catccccccac caatccatag
aaatactgtc ttccacaaaa atgatccctg gtgccaaaaa tgttagagac cactcccctа aaactctctt cttagctctc acctcctgta
ttactatctc atctcagtac attgaagccc ccatcttttc cccatggatg cctcatttcc tattagggag gcatttttтt atttttgtt tttatttttt
tccgagacgg agtctcgctc tgtcgccaag gctggagtgc agtggcgcga tctcggctca ctgcaagctc cgcctcccgg gttcacgcca
ttctcctgcc tcagcctccc aagtagctgg gactacaggc gcccgcacta cgcccggcta atttttgta tttttagtag agacggggtt
tcaccgtggt agccaggatg gtctcgatct cctgacctcg tgatccgccc gccttggcct cccaaagtgc tgggattaca ggcgtgagac
cgcgcccggc cgtcatttgg tatgtcttaa tgtgcctcag gacctagcac agtccctggt acccagtaga gacctatgta atgttcgtta
ttcaataata aatacatgaa ttaaagagtg agagtggatt ttgtaatgtt acgactgata gagaaatact cagtgattct aagggatggg
gaagaacggt tggagctaga ggttgtgctc aggaaactat taaatagacg ttccgcagga agggattgac gaagtgtgag gttaatgagg
aagggaaaat agaatataaa atttggtggt ggaaaagatc tgattcatga tgccgtgtca gagagcaaag ctcctgtcct tttggcctaa
tttggtgatg ctgttcttgg gtctaccaca cctccttttg ccctccgcag gagcctgtgt tggaagagat ggtgatgggc ctgggcgttt tgttgttggt
cttcgtgctg ggtctgggtc tgaccccacc gaccctggct caggataact ccaggtacac acacttcctg acccagcact atgatgccaa
accacagggc cgggatgaca gatactgtga aagcatcatg aggagacggg gcctgacctc accctgcaaa gacatcaaca catttattca
tggcaacaag cgcagcatca aggccatctg tgaaaacaag aatggaaacc ctcacagaga aaacctaaga ataagcaagt cttctttcca
ggtcaccact tgcaagctac atggaggttc cccctggcct ccatgccagt accgagccac agcgggttc agaaacgttg ttgttgcttg
tgaaaatggc ttacctgtcc acttggatca gtcaattttc cgtcgtccgt aaccagcggg cccctggtca agtgctggct ctgctgtcct
tgccttccat ttcccctctg cacccagaac agtggtggca acattcattg ccaagggccc aaagaaagag ctacctggac cttttgtttt
ctgtttgaca acatgtttaa taaataaaaa tgtcttgata tcagtaagaa tcagagtctt ctcactgatt ctgggcatat tgatctttcc cccattttct
ctacttggct gctccctgag aggactgcat aggatagaaa tgccttttтc ttttctttтc gttttttttt ttttttttt ttgagatgga gtctcactct
gtcgcccagg cttaagtgca atggcacaat ctcggctcac tgcaacctct ctctcctggg ttcaagtgat tctcctgcct cagcctccca
aatagctgag attacaggca tgcaccacca cacctggcta atttttgtgt ttttagtaga gacagggttt caccgttttg gccaggttgg
tcttgaactc ctgacctcgg gagatccgcc caccttggcc tctctttgtg ctgggattac aggcatgagc cactgagccg ggccactttt
tccttatcag tcagttttta caagtcatta gggaggtaga ctttacctct ctgtgaagga aagtatggta tgttgatcta cagagagaga
tggaaaaatt ccagggctcg tagctactaa gcagaatttc caagataggc aaattgtttt ttctgtcaaa taataagcta atattacttc
tacaaatatg agaccttgga gagaagtttc caaggaccaa gtaccaacat accaacagat tattatagtt tctctcactc ttacacacac
acacacacat atacacatat gtaatccagc atgaatacca aaattcattc agggtagcca ccttttgtct taatcgagag ataattttga
tgtttgaatg gaatgctccc aggatattct cttgtcatgg ttattttata taaaattcaa aaaccaatta cattatttcc tctgtaatct tttactttat
caactaatgt ctggcaagtg tgatgttttg gggaagttat agaagattcc ggccaggcgc ttatctcacg cttgtaatcc agcacttttgg
gaagctgagg cggacagatc acgaggtcaa gagatcaaga ccatcctgga caacatggtg aaaccttgtc tctactaaaa atgtgaaaat
tagctgggcg tggtggcaca cacctatagt cccagctact cgggaggctg aggcaggaga atcgcttgaa cctaggaggc ggaggttgca

Figure 10B (Continued)

ctgagccgag atcacgccac tgcactccag cctgggcgac agagcgagac tccatctcaa aaaaaaaaaa aaaagaaaga tcccagttta tcccagttta tcccttattc ttcctcaatt ctcaagattt gtttttaagt taacataact taggttaaca cactctttgt aaaatacact gttcaatcta cagactcagt ggttagcttc ctgttaacta atttctgttg acaggtactt ggatatttta tttagaaagt ggttgccaat aaattagtta taagtcgcca gtttcactgc cttgtgaaca cataattatt gtggtctcag tattccctat ggtggcttct cctgctcctg gtattgccct gaaatgggcc aaaagccgtg gctccccaat gctcaggtta tagaacattg tccaggtacc acctaggaga gcccagcctc actgaaagta ttcaaattta ggaatgggtt tgagaagtag gtagctggta tgtgcttagc acaagaatct ctcttccttg ggttagtctg tttcaaaact gaaaacactg tcattcctta agaaaatagg aaaaagtatt ccaaacctct gtcactagaa aatttgccat attaccaaat ctcaaaaacc tctcaggaaa tgagaaagtc ccagtttctg gtaaactatt tgggcccttt tctcaagttc tccttccagt gctatttcct tgaggtgagg caaagttact caagatcatc gctgccactc aaggccttga tagggcaagt gaaaggcatg gaccattatt atattgatca cagcataagc tgtgaaaacc cacatcttct ccaaacatct gcttggagca ttatcatcgc atagtttgct ctggtgttca gggaaatcgc tgtttcatag gaaatcacat ggcagtggga tgggagtgtt tcctgacctg ccgatggtac tggcacctga gcaagcattc ctagtccttt ttggtctggg cctcttgttc tatcacaacc acaagctgtt taaaataaaa acgtcaagtc acaggcaggt cattttatcc tgcgtgaatc aattgaag

Figure 11A

MWVLFLLSGLGGLRMDSNFDSLPVQITVPEKIRSIIKEGIESQASYKIVIEGKPYTVNLMQKNFLPH
NFRVYSYSGTGIMKPLDQDFQNFCHYQGYIEGYPKSVVMVSTCTGLRGVLQFENVSYGIEPLES
SVGFEHVIYQVKHKKADVSLYNEKDIESRDLSFKLQSAEPQQDFAKYIEMHVIVEKQLYNHMGSD
TTVVAQKVFQLIGLTNAIFVSFNITIILSSLELWIDENKIATTGEANELLHTFLRWKTSYLVLRPHDVA
FLLVYREKSNYVGATFQGKMCDANYAGGVVLHPRTISLESLAVILAQLLSLSMGITYDDINKCQCS
GAVCIMNPEAIHFSGVKIFSNCSFEDFAHFISKQKSQCLHNQPRLDPFFKQQAVCGNAKLEAGEE
CDCGTEQDCALIGETCCDIATCRFKAGSNCAEGPCCENCLFMSKERMCRPSFEECDLPEYCNG
SSASCPENHYVQTGHPCGLNQWICIDGVCMSGDKQCTDTFGKEVEFGPSECYSHLNSKTDVSG
NCGISDSGYTQCEADNLQCGKLICKYVGKFLLQIPRATIIYANISGHLCIAVEFASDHADSQKMWIK
DGTSCGSNKVCRNQRCVSSSYLGYDCTTDKCNDRGVCNNKKHCHCSASYLPPDCSVQSDLWP
GGSIDSGNFPPVAIPARLPERRYIENIYHSKPMRWPFFLFIPFFIIFCVLIAIMVKVNFQRKKWRTED
YSSDEQPESESEPKG

Figure 11B

catctcgcac ttccaactgc cctgtaacca ccaactgccc ttattccggc tgggacccag gacttcaagc catgtgggtc ttgtttctgc
tcagcgggct cggcgggctg cggatggaca gtaattttga tagtttacct gtgcaaatta cagttccgga gaaaatacgg tcaataataa
aggaaggaat tgaatcgcag gcatcctaca aaattgtaat tgaagggaaa ccatatactg tgaatttaat gcaaaaaaac tttttacccc
ataattttag agtttacagt tatagtggca caggaattat gaaaccactt gaccaagatt ttcagaattt ctgccactac caagggtata
ttgaaggtta tccaaaatct gtggtgatgg ttagcacatg tactggactc aggggcgtac tacagtttga aaatgttagt tatggaatag
aacccctgga gtcttcagtt ggctttgaac atgtaattta ccaagtaaaa cataagaaag cagatgtttc cttatataat gagaaggata
ttgaatcaag agatctgtcc tttaaattac aaagcgcaga gccacagcaa gattttgcaa agtatataga aatgcatgtt atagttgaaa
aacaattgta taatcatatg gggtctgata caactgttgt cgctcaaaaa gttttccagt tgattggatt gacgaatgct atttttgttt catttaatat
tacaattatt ctgtcttcat tggagctttg gatagatgaa aataaaattg caaccactgg agaagctaat gagttattac acacattttt
aagatggaaa acatcttatc ttgttttacg tcctcatgat gtggcatttt tacttgttta cagagaaaag tcaaattatg ttggtgcaac ctttcaaggg
aagatgtgtg atgcaaacta tgcaggaggt gttgttctgc accccagaac cataagtctg gaatcacttg cagttatttt agctcaatta
ttgagcctta gtatggggat cacttatgat gacattaaca aatgccagtg ctcaggagct gtctgcatta tgaatccaga agcaattcat
ttcagtggtg tgaagatctt tagtaactgc agcttcgaag actttgcaca ttttatttca aagcagaagt cccagtgtct tcacaatcag cctcgcttag
atccttttt caaacagcaa gcagtgtgtg gtaatgcaaa gctggaagca ggagaggagt gtgactgtgg gactgaacag gattgtgccc
ttattggaga aacatgctgt gatattgcca catgtagatt taaagccggt tcaaactgtg ctgaaggacc atgctgcgaa aactgtctat
ttatgtcaaa agaaagaatg tgtaggcctt cctttgaaga atgcgacctc cctgaatatt gcaatggatc atctgcatca tgcccagaaa
accactatgt tcagactggg catccgtgtg gactgaatca atggatctgt atagatggag tttgtatgag tggggataaa caatgtacag
acacatttgg caaagaagta gagtttggcc cttcagaatg ttattctcac cttaattcaa agactgatgt atctggaaac tgtggtataa
gtgattcagg atacacacag tgtgaagctg acaatctgca gtgcggaaaa ttaatatgta aatatgtagg taaattttta ttacaaattc
caagagccac tattatttat gccaacataa gtggacatct ctgcattgct gtggaatttg ccagtgatca tgcagacagc caaaagatgt
ggataaaaga tggaacttct tgtggttcaa ataaggtttg caggaatcaa agatgtgtga gttcttcata cttgggttat gattgtacta
ctgacaaatg caatgataga ggtgtatgca ataacaaaaa gcactgtcac tgtagtgctt catatttacc tccagattgc tcagttcaat
cagatctatg gcctggtggg agtattgaca gtggcaattt tccacctgta gctataccag ccagactccc tgaaaggcgc tacattgaga
acatttacca ttccaaacca atgagatggc catttttctt attcattcct ttctttatta ttttctgtgt actgattgct ataatggtga aagttaattt
ccaaaggaaa aaatggagaa ctgaggacta ttcaagcgat gagcaacctg aaagtgagag tgaacctaaa gggtagtctg gacaacagag
atgccatgat atcacttctt ctagagtaat tatctgtgat ggatggacac aaaaaaatgg aaagaaaaga atgtacatta cctggtttcc
tgggattcaa acctgcatat tgtgatttta atttgaccag aaaatatgat atatatgtat aatttcacag ataatttact tatttaaaaa tgcatgataa
tgagttttac attacaaatt tctgtttttt taaagttatc ttacgctatt tctgttggtt agtagacact aattctgtca gtaggggcat ggtataagga
aatatcataa tgtaatgagg tggtactatg attaaaagcc actgttacat ttcaaaaaaa aaaaaaaaaa

FIGURE 12

agagngtcgcccctttttttttttttttttttttttttttttttttttttttttgacatttataaatgaacctttattaaagacacttcaatgccatttgttanacacttcaatattttaca
tggttttcaatgtacactgtaccaaaatttctataaataaataactttgtacataaaagtaatactccctctttcacattgcctcncagaagcagcaaattcatatattt
tgtggaagtaagattagtcagttaactgtcaagaacaaaattctaaatgtgcttacctttttgaacagtgatgacacctgacagtaattgttaactatttttctcagtaa
ctcccttcagcttttggccaaaggaacatttgaaggaccttgtttcnatttaagttttactaaatgacacattggcactcanaanatggttagctaccagtctcaaa
agtgcaaattatacccanaacccaggtcaagggctgtcctttccaagtcccagctcagtttcatctggtgcgaaggaatggcatggacaggcctgctccggg
tccttaatanaaataaggtancectgaaaagtcanaacttcctcctttctgtcccccaagggcaatgtaatactcattanattgggcaaaacnaaaacatcngt
atagtaaaaatccacaggtnccaacaccagcagcctttaccttantttnaaaggccncaaaatagca

Figure 13A

MSPHLTALLGLVLCLAQTIHTQEGALPRPSISAEPGTVISPGSHVTFMCRGPVGVQTFRLEREDR
AKYKDSYNVFRLGPSESEARFHIDSVSEGNAGLYRCLYYKPPGWSEHSDFLELLVKGTVPGTEA
SGFDAP

Figure 13B

ccacgcgtcc ggggaccggg gccatgtctc cacacctcac tgctctcctg ggcctagtgc tctgcctggc ccagaccatc cacacgcagg aggggggcct tcccagaccc tccatctcgg ctgagccagg cactgtgatc tccccgggga gccatgtgac tttcatgtgc cggggcccgg ttggggttca aacattccgc ctggagaggg aggatagagc caagtacaaa gatagttata atgtgtttcg acttggtcca tctgagtcag aggccagatt ccacattgac tcagtaagtg aaggaaatgc cgggctttat cgctgcctct attataagcc ccctggatgg tctgagcaca gtgacttcct ggagctgctg gtgaaaggga ctgtgccagg cactgaagcc tccggatttg atgcaccatg aatgaggaga aatggcctcc cgtcttgtga acttcaatgg ggagaaataa ttagaatgag caatagaaat gcacagatgc ctatacatac atatacaaat aaaaagatac gattcgcaaa aaaaaaaaaa aaaagggc

Figure 14A

MPLLWLRGFLLASCWIIVRSSPTPGSEGHSAAPDCPSCALAALPKDVPNSQPEMVEAVKKHILN
MLHLKKRPDVTQPVPKAALLNAIRKLHVGKVGENGYVEIEDDIGRRAEMNELMEQTSEIITFAESG
TARKTLHFEISKEGSDLSVVERAEVWLFLKVPKANRTRTKVTIRLFQQQKHPQGSLDTGEEAEEV
GLKGERSELLLSEKVVDARKSTWHVFPVSSSIQRLLDQGKSSLDVRIACEQCQESGASLVLLGKK
KKKEEEGEGKKKGGGEGGAGADEEKEQSHRPFLMLQARQSEDHPHRRRRRGLECDGKVNICC
KKQFFVSFKDIGWNDWIIAPSGYHANYCEGECPSHIAGTSGSSLSFHSTVINHYRMRGHSPFANL
KSCCVPTKLRPMSMLYYDDGQNIIKKDIQNMIVEECGCS

Figure 14B

tccacacaca caaaaaacct gcgcgtgagg ggggaggaaa agcagggcct ttaaaaaggc aatcacaaca acttttgctg ccaggatgcc cttgctttgg ctgagaggat ttctgttggc aagttgctgg attatagtga ggagttcccc caccccagga tccgaggggc acagcgcggc ccccgactgt ccgtcctgtg cgctggccgc cctcccaaag gatgtaccca actctcagcc agagatggtg gaggccgtca agaagcacat tttaaacatg ctgcacttga agaagagacc cgatgtcacc cagccggtac ccaaggcggc gcttctgaac gcgatcagaa agcttcatgt gggcaaagtc ggggagaacg ggtatgtgga gatagaggat gacattggaa ggagggcaga aatgaatgaa cttatggagc agacctcgga gatcatcacg tttgccgagt caggaacagc caggaagacg ctgcacttcg agatttccaa ggaaggcagt gacctgtcag tggtggagcg tgcagaagtc tggctcttcc taaaagtccc caaggccaac aggaccagga ccaaagtcac catccgcctc ttccagcagc agaagcaccc gcagggcagc ttggacacag gggaagaggc cgaggaagtg ggcttaaagg gggagaggag tgaactgttg ctctctgaaa aagtagtaga cgctcggaag agcacctggc atgtcttccc tgtctccagc agcatccagc ggttgctgga ccagggcaag agctccctgg acgttcggat tgcctgtgag cagtgccagg agagtggcgc cagcttggtt ctcctgggca agaagaagaa gaaagaagag gaggggaag ggaaaaagaa gggcggaggt gaaggtgggg caggagcaga tgaggaaaag gagcagtcgc acagaccttt cctcatgctg caggcccggc agtctgaaga ccaccctcat cgccggcgtc ggcggggctt ggagtgtgat ggcaaggtca acatctgctg taagaaacag ttctttgtca gtttcaagga catcggctgg aatgactgga tcattgctcc ctctggctat catgccaact actgcgaggg tgagtgcccg agccatatag caggcacgtc cgggtcctca ctgtccttcc actcaacagt catcaaccac taccgcatgc ggggccatag ccccttgcc aacctcaaat cgtgctgtgt gcccaccaag ctgagaccca tgtccatgtt gtactatgat gatggtcaaa acatcatcaa aaaggacatt cagaacatga tcgtggagga gtgtgggtgc tcatagagtt gcccagccca gggggaaagg gagcaagagt tgtccagaga agacagtggc aaaatgaaga aatttttaag gtttctgagt taaccagaaa aatagaaatt aaaaacaaaa caaaacaaaa aaaaaaacaa aaaaaaacaa aagtaaatta aaaacaaacc tgatgaaaca gatgaaacag atgaaggaag atgtggaaat cttagcctgc cttagccagg gctcagagat gaagcagtga agagacagat tgggagggaa agggagaatg gtgtaccctt tatttcttct gaaatcacac tgatgacatc agttgtttaa acggggtatt gtcctttccc ccttgaggt tcccttgtga gcttgaatca accaatctga tctgcagtag tgtggactag aacaacccaa atagcatcta gaaagccatg agtttgaaag ggcccatcac aggcactttc ctagcctaat

Figure 15A

MNCVCRLVLVVLSLWPDTAVAPGPPPGPPRVSPDPRAELDSTVLLTRSLLADTRQLAAQLRDKF
PADGDHNLDSLPTLAMSAGALGALQLPGVLTRLRADLLSYLRHVQWLRRAGGSSLKTLEPELGT
LQARLDRLLRRLQLLMSRLALPQPPPDPPAPPLAPPSSAWGGIRAAHAILGGLHLTLDWAVRGLL
LLKTRL

Figure 15B

gaagggttaa aggcccccgg ctccctgccc cctgccctgg ggaacccctg gccctgtggg gacatgaact gtgtttgccg cctggtcctg gtcgtgctga gcctgtggcc agatacagct gtcgcccctg ggccaccacc tggcccccct cgagtttccc cagaccctcg ggccgagctg gacagcaccg tgctcctgac ccgctctctc ctggcggaca cgcggcagct ggctgcacag ctgagggaca aattcccagc tgacggggac cacaacctgg attccctgcc caccctggcc atgagtgcgg gggcactggg agctctacag ctcccaggtg tgctgacaag gctgcgagcg gacctactgt cctacctgcg gcacgtgcag tggctgcgcc gggcaggtgg ctcttccctg aagaccctgg agcccgagct gggcaccctg caggcccgac tggaccggct gctgcgccgg ctgcagctcc tgatgtcccg cctggccctg ccccagccac ccccggaccc gccggcgccc ccgctggcgc ccccctcctc agcctggggg ggcatcaggg ccgcccacgc catcctgggg gggctgcacc tgacacttga ctgggccgtg aggggactgc tgctgctgaa gactcggctg tgacccgggg cccaaagcca ccaccgtcct tccaaagcca gatcttattt atttatttat ttcagtactg ggggcgaaac agccaggtga tccccccgcc attatctccc cctagttaga gacagtcctt ccgtgaggcc tgggggacat ctgtgcctta tttatactta tttatttcag gagcaggggt gggaggcagg tggactcctg ggtccccgag gaggagggga ctggggtccc ggattcttgg gtctccaaga agtctgtcca cagacttctg ccctggctct tccccatcta ggcctgggca ggaacatata ttatttattt aagcaattac ttttcatgtt ggggtgggga cggaggggaa agggaagcct gggtttttgt acaaaaatgt gagaaacctt tgtgagacag agaacaggga attaaatgtg tcatacatat ccacttgagg gcgatttgtc tgagagctgg ggctggatgc ttgggtaact ggggcagggc aggtggaggg gagacctcca ttcaggtgga ggtcccgagt gggcggggca gcgactggga gatgggtcgg tcacccagac agctctgtgg aggcagggtc tgagccttgc ctggggcccc gcactgcata gggccgtttg tttgtttttt gagatggagt ctcgctctgt tgcctaggct ggagtgcagt gaggcaatct aaggtcactg caagctccac ctcccgggtt caagcaattc tcctgcctca gcctcccgat tagctgggat cacaggtgtg caccaccatg cccagctaat tatttatttc ttttgtattt ttagtagaga cagggtttca ccatgttggc caggctggtt tcgaactcct gacctcaggt gatcctcctg cctcggcctc ccaaagtgct gggattacag gtgtgagcca ccacacctga cccataggtc ttcaataaat atttaatgga aggttccaca agtcaccctg tgatcaacag tacccgtatg ggacaaagct gcaaggtcaa gatggttcat tatggctgtg ttcaccatag caaactggaa agaatctaga tatccaacag tgagggttaa gcaacatggt gcatctgtgg atagaacacc acccagccgc ccggagcagg gactgtcatt cagggaggct aaggagagag gcttgcttgg gatatagaaa gatatcctga cattggccag gcatggtggc tcacgcctgt aatcctggca ctttgggagg acgaagcgag tggatcactg aagtccaaga gtttgagacc ggcctgcgag acatggcaaa accctgtctc aaaaaagaaa gaatgatgtc ctgacatgaa acagcaggct acaaaaccac tgcatgctgt gatcccaatt ttgtgttttt ctttctatat atggattaaa acaaaaatcc taaagggaaa tacgccaaaa tgttgacaat gactgtctcc aggtcaaagg agagaggtgg gattgtgggt gacttttaat gtgtatgatt gtctgtattt tacagaattt ctgccatgac tgtgtatttt gcatgacaca ttttaaaaat aataaacact atttttagaa t

Figure 16A

MSPNFKLQCHFILIFLTALRGESRYLELREAADYDPFLLFSANLKRDVAGEQPYRRALRCLDMLSL
QGQFTFTADRPQLHCAAFFISEPEEFITIHYDQVSIDCQGGDFLKVFDGWILKGEKFPSSQDHPLP
SAERYIDFCESGLSRRSIRSSQNVAMIFFRVHEPGNGFTLTIKTDPNLFPCNVISQTPNGKFTLVV
PHQHRNCSFSIIYPVVIKISDLTLGHVNGLQLKKSSAGCEGIGDFVELLEGTGLDPSKMTPLADLC
YPFHGPAQMKVGCDNTVVRMVSSGKHVNRVTFEYRQLEPYELENPNGNSIGEFCLSGL

Figure 16B

ggacctccgg agcagacagc acagcagctg cagaggcaag gccagcatgt cgcccaactt caaacttcag tgtcacttca ttctcatctt cctgacggct ctaagagggg aaagccggta cctagagctg agggaagcgg cggactacga tcctttcctg ctcttcagcg ccaacctgaa gcgggacgtg gctggggagc agccgtaccg ccgcgctctg cggtgcctgg acatgctgag cctccagggc cagttcacct tcaccgccga ccggccgcag ctgcactgcg cagccttctt catcagcgag cccgaggagt tcattaccat ccactacgac caggtctcca tcgactgtca gggcggcgac ttcctgaagg tatttgatgg ttggattctc aaggggggaga agttccccag ttcccaggat catcctctcc cctcagctga gcggtacata gatttctgtg agagtggtct tagcaggagg agcatcagat cttcccagaa tgtggccatg atcttcttcc gagtccatga accaggaaat ggattcacat taaccataaa gacagacccc aacctctttc cttgcaatgt catttctcag actccaaatg gaaagtttac cctggtagtt ccacaccagc atcgaaactg cagcttctcc ataatttatc ctgtggtgat caaaatatct gatcttaccc tgggacacgt aaatggtctt cagttaaaga aatcctcagc aggttgcgag ggaataggag actttgtgga gctgctggag ggaactggat tggacccttc caagatgacg cctttagctg atctctgcta ccccttttcat ggcccggccc agatgaaagt tggctgtgac aacactgtgg tgcgcatggt ctccagtgga aaacacgtaa atcgtgtgac ttttgagtat cgtcagctgg agccgtacga gctggaaaac ccaaatggaa acagtatcgg ggaattctgt ttgtctggtc tttgaataac caacccagtg atttacatgc tgatagctaa gtgagttttt aatggccatt gtgtatgatt ttgatgcaca actagttaaa agcctttcat accagtcagt atttcccagc cttgagcgca cgcacacacc acacacatac acacacgcat tatttttgtt actttgcttc tttttatgtt tgtaatctgt aaatgaacac atggcagaaa ataaccctga ttggtagg

Figure 17A

TTPDRRLWNPPATSSSLRQMERMLPLLTLGLLAAGFCPAVLCHPNSPLDEENLTQENQDRGTH
VDLGLASANVDFAFSLYKQLVLKAPDKNVIFSPLSISTALAFLSLGAHNTTLTEILKGLKFNLTETSE
AEIHQSFQHLLRTLNQSSDELQLSMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAA
KKLINDYVKNGTRGKITDLIKDLDSQTMMVLVNYIFFKAKWEMPFDPQDTHQSRFYLSKKKWVM
VPMMSLHHLTIPYFRDEELSCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEF
REIGELYLPKFSISRDYNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEA
SAATAVKITLLSALVETRTIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA

Figure 17B

ctgtctcaaa ataaaaataa aaaataaaaa gaaataaaaa agaaatatac caaaatgtta gctggggtct tctctgggta gtaaagtgct gggggatatt ttccaaagtc cttctttaca ttctctgagt ttttccatgt tcttcaatga gtatttaata agcagataaa aactaataca acaaaggatt ttttctgtgt gctttttga cctttggagg aagagattag agctagtccc ataaccaggt tatttgagta ggtctaataa gcccgtatta ccagaaatta tcatctggtc atttccagtc cgagaacaga acacttggtt gtcctggcat ttcccaagca gtgggaggag ttctctgcag gaataaataa gcctcagcat tcatgaaaat ccactactcc agacagacgg ctttggaatc caccagctac atccagctcc ctgaggcagg taatccatga tgttttacat cctgggagcg gaggaatctg tttttccagg agagttttag gcagcagcct ggagtgtgtg gagtgtgagg ggtaagcaga g

Figure 18A

MVLLTAVLLLLAAYAGPAQSLGSFVHCEPCDEKALSMCPPSPLGCELVKEPGCGCCMTCALAEG
QSCGVYTERCAQGLRCLPRQDEEKPLHALLHGRGVCLNEKSYREQVKIERDSREHEEPTTSEM
AEETYSPKIFRPKHTRISELKAEAVKKDRRKKLTQSKFVGGAENTAHPRIISAPEMRQESEQGPC
RRHMEASLQELKASPRMVPRAVYLPNCDRKGFYKRKQCKPSRGRKRGICWCVDKYGMKLPGM
EYVDGDFQCHTFDSSNVE

Figure 18B

tgaaaaaaaa aaaaggaaag aaagggattg aaggagcttg ccaagggtag gctgcctaaa ttcacatttt ccctgggtct ttccgtgaaa tggggacacc agaaacccaa gggtcgggtc tagtgccctc aactctctgg ggatgagagt cttgccttgg ggtagacaag aggcagggca gggaggagca gagccctggg gtgcggccgt cctcaccgcc tgttgctcta ctcaccccag tgcaaacctt cccgtggccg caagcgtggc atctgctggt gcgtggacaa gtacgggatg aagctgccag gcatggagta cgttgacggg gactttcagt gccacacctt cgacagcagc aacgttgagt gatgcgtccc cccccaacct ttccctcacc ccctcccacc cccagccccg actccagcca gcgcctcccc ccaccccagg acgccactca tttcatctca tttaagggaa aaatatatat ctatctattt gaggaaactg aggacctcgg aatctctagc aagggctcaa cttcgaaaat ggcaacaaca gagatgcaaa aagctaaaaa gacacccccc ccctttaaat ggttttcttt ttgaggcaag ttggatgaac agagaaggga agagaggaag aacgagagga agagaaggga aggaagtgtt tgtgtagaag agagagaaag acgaatagag ttaggaaaag gaagacaagc aggtgggcag gaaggacatg caccgagacc aggcaggggc ccaactttca cgtccagccc tggcctgggg tcgggagagg tgggcgctag aagatgcagc ccaggatgtg gcaatcaatg acactattgg ggtttcccag gatggattgg tcaggggggag aaaggaaaag gcaaaacact ccaggacctc tcccggatct gtctcctcct ctagccagca gtatggacag ctggaccccct gaacttcctc tcctcttacc tgggcagagt gttgtctctc cccaaattta taaaaactaa aatgcattcc attcctctga aagcaaaaca aattcataat tgagtgatat taaatagaga ggttttcgga agcagatctg tgaatatgaa atacatgtgc atatttcatt ccccaggcag acatttttta gaaatcaata catgccccaa tattggaaag acttgttctt ccacggtgac tacagtacat gctgaagcgt gccgtttcag ccctcattta attcaatttg taagtagcgc acgagcctct gtgggggagg ataggctgaa aaa

Figure 19

MVLIQIPMYN EKEVCQLSIG AACRLSWPLD RMIVQVLDDS TDPASKELVN AECDKWARKG
INIMSEIRDN RIGYKAGALK AGMMHNYVKQ CEFVAIFDAD FQPDPDFLER TIPFLIHNHE
ISLVQCRWKF VNANECLMTR MQEMSLNYHF VAEQESGSSI HAFFGFNGTA GVWRIAALNE
AGGWKDRTTV EDMDLAVRAC LHGWKFVYVH DVEVKNELPS TFKAYRFQQH
RWSCGPANLW RKMTMEILQN KKVSAWKKLY LIYNFFFIRK IVVHIFTFVF YCLILPTTVL
FPELQVPKWA TVYFPTTITI LNAIATPRMI KSLTYIVYCR SLHLLVFWIL FENVMSMHRT
KATFIGLLEA GRVNEWVVTE KLGDTLKSKL IGKATTKLYT RFGQRLNWRE LVVGLYIFFC
GCYDFAYGGS YFYVYLFLQS CAFFVAGVGY IGTFVPTV

Figure 20A

MPAGRRGPAAQSARRPPPLLPLLLLLCVLGAPRAGSGAHTAVISPQDPTLLIGSSLLATCSVHGD
PPGATAEGLYWTLNGRRLPPELSRVLNASTLALALANLNGSRQRSGDNLVCHARDGSILAGSCL
YVGLPPEKPVNISCWSKNMKDLTCRWTPGAHGETFLHTNYSLKYKLRWYGQDNTCEEYHTVGP
HSCHIPKDLALFTPYEIWVEATNRLGSARSDVLTLDILDVVTTDPPPDVHVSRVGGLEDQLSVRW
VSPPALKDFLFQAKYQIRYRVEDSVDWKVVDDVSNQTSCRLAGLKPGTVYFVQVRCNPFGIYGS
KKAGIWSEWSHPTAASTPRSERPGPGGGACEPRGGEPSSGPVRRELKQFLGWLKKHAYCSNL
SFRLYDQWRAWMQKSHKTRNQDEGILPSGRRGTARGPAR

Figure 20B

cgcccagcga cgtgcgggcg gcctggcccg cgccctcccg cgcccggcct gcgtcccgcg ccctgcgcca ccgccgccga gccgcagccc gccgcgcgcc cccggcagcg ccggccccat gcccgccggc cgccggggcc ccgccgccca atccgcgcgg cggccgccgc cgttgctgcc cctgctgctg ctgctctgcg tcctcggggc gccgcgagcc ggatcaggag cccacacagc tgtgatcagt ccccaggatc ccacgcttct catcggctcc tccctgctgg ccacctgctc agtgcacgga gacccaccag gagccaccgc cgagggcctc tactggaccc tcaacgggcg ccgcctgccc cctgagctct cccgtgtact caacgcctcc accttggctc tggccctggc caacctcaat gggtccaggc agcggtcggg ggacaacctc gtgtgccacg cccgtgacgg cagcatcctg gctggctcct gcctctatgt tggcctgccc ccagagaaac ccgtcaacat cagctgctgg tccaagaaca tgaaggactt gacctgccgc tggacgccag gggcccacgg ggagaccttc ctccacacca actactccct caagtacaag cttaggtggt atggccagga caacacatgt gaggagtacc acacagtggg gccccactcc tgccacatcc ccaaggacct ggctctcttt acgccctatg agatctgggt ggaggccacc aaccgcctgg gctctgcccg ctccgatgta ctcacgctgg atatcctgga tgtggtgacc acggaccccc cgcccgacgt gcacgtgagc cgcgtcgggg gcctggagga ccagctgagc gtgcgctggg tgtcgccacc cgccctcaag gatttcctct ttcaagccaa ataccagatc cgctaccgag tggaggacag tgtggactgg aaggtggtgg acgatgtgag caaccagacc tcctgccgcc tggccggcct gaaacccggc accgtgtact tcgtgcaagt gcgctgcaac cctttggca tctatggctc caagaaagcc gggatctgga gtgagtggag ccaccccaca gccgcctcca ctccccgcag tgagcgcccg ggcccgggcg gcggggcgtg cgaaccgcgg ggcggagagc cgagctcggg gccggtgcgg cgcgagctca agcagttcct gggctggctc aagaagcacg cgtactgctc caacctcagc ttccgcctct acgaccagtg gcgagcctgg atgcagaagt cgcacaagac ccgcaaccag gacgagggga tcctgccctc gggcagacgg ggcacggcga gaggtcctgc cagataagct gtaggggctc aggccaccct ccctgccacg tggagacgca gaggccgaac ccaaactggg gccacctctg taccctcact tcagggcacc tgagccaccc tcagcaggag ctggggtggc ccctgagctc caacggccat aacagctctg actcccacgt gaggccacct ttgggtgcac cccagtgggt gtgtgtgtgt gtgtgagggt tggttgagtt gcctagaacc cctgccaggg ctgggggtga gaaggggagt cattactccc cattacctag ggcccctcca aaagagtcct tttaaataaa tgagctattt aggtgc

Figure 21

MLHVEMLTLV FLVLWMCVFS QDPGSKAVAD RYAVYWNSSN PRFQRGDYHI DVCINDYLDV
FCPHYEDSVP EDKTERYVLY MVNFDGYSAC DHTSKGFKRW ECNRPHSPNG PLKFSEKFQL1
FTPFSLGFEF RPGREYFYIS SAIPDNGRRS CLKLKVFVRP TNSCMKTIGV HDRVFDVNDK
VENSLEPADD TVHESAEPSR GENAAQTPRI PSRLLAILLF LLAMLLTL

Figure 22A

MGGCTVKPQLLLLALVLHPWNPCLGADSEKPSSIPTDKLLVITVATKESDGFHRFMQSAKYFNYT
VKVLGQGEEWRGGDGINSIGGGQKVRLMKEVMEHYADQDDLVVMFTECFDVIFAGGPEEVLKK
FQKANHKVVFAADGILWPDKRLADKYPVVHIGKRYLNSGGFIGYAPYVNRIVQQWNLQDNDDDQ
LFYTKVYIDPLKREAINITLDHKCKIFQTLNGAVDEVVLKFENGKARAKNTFYETLPVAINGNGPTKI
LLNYFGNYVPNSWTQDNGCTLCEFDTVDLSAVDVHPNVSIGVFIEQPTPFLPRFLDILLTLDYPKE
ALKLFIHNKEVYHEKDIKVFFDKAKHEIKTIKIVGPEENLSQAEARNMGMDFCRQDEKCDYYFSVD
ADVVLTNPRTLKILIEQNRKIIAPLVTRHGKLWSNFWGALSPDGYYARSEDYVDIVQGNRVGVWN
VPYMANVYLIKGKTLRSEMNERNYFVRDKLDPDMALCRNAREMGVFMYISNRHEFGRLLSTANY
NTSHYNNDLWQIFENPVDWKEKYINRDYSKIFTENIVEQPCPDVFWFPIFSEKACDELVEEMEHY
GKWSGGKHHDSRISGGYENVPTDDIHMKQVDLENVWLDFIREFIAPVTLKVFAGYYTKGFALLNF
VVKYSPERQRSLRPHHDASTFTINIALNNVGEDFQGGGCKFLRYNCSIESPRKGWSFMHPGRLT
HLHEGLPVKNGTRYIAVSFIDP

Figure 22B

atgggggat gcacggtgaa gcctcagctg ctgctcctgg cgctcgtcct ccacccctgg aatccctgtc tgggtgcgga ctcggagaag ccctcgagca tccccacaga taaattatta gtcataactg tagcaacaaa agaaagtgat ggattccatc gatttatgca gtcagccaaa tatttcaatt atactgtgaa ggtccttggt caaggagaag aatggagagg tggtgatgga attaatagta ttggaggggg ccagaaagtg agattaatga aagaagtcat ggaacactat gctgatcaag atgatctggt tgtcatgttt actgaatgct ttgatgtcat atttgctggt ggtccagaag aagttctaaa aaaattccaa aaggcaaacc acaaagtggt ctttgcagca gatggaattt tgtggccaga taaaagacta gcagacaagt atcctgttgt gcacattggg aaacgctatc tgaattcagg aggatttatt ggctatgctc catatgtcaa ccgtatagtt caacaatgga atctccagga taatgatgat gatcagctct tttacactaa agtttacatt gatccactga aaagggaagc tattaacatc acattggatc acaaatgcaa aattttccag accttaaatg gagctgtaga tgaagttgtt ttaaaatttg aaaatggcaa agccagagct aagaatacat tttatgaaac attaccagtg gcaattaatg gaaatggacc caccaagatt ctcctgaatt attttggaaa ctatgtaccc aattcatgga cacaggataa tggctgcact ctttgtgaat tcgatacagt cgacttgtct gcagtagatg tccatccaaa cgtatcaata ggtgtttttа ttgagcaacc aacccctttt ctacctcggt ttctggacat attgttgaca ctggattacc caaaagaagc acttaaactt tttattcata acaaagaagt ttatcatgaa aaggacatca aggtattttt tgataaagct aagcatgaaa tcaaaactat aaaaatagta ggaccagaag aaaatctaag tcaagcggaa gccagaaaca tgggaatgga cttttgccgt caggatgaaa agtgtgatta ttactttagt gtggatgcag atgttgtttt gacaaatcca aggactttaa aaattttgat tgaacaaaac agaaagatca ttgctcctct tgtaactcgt catggaaagc tgtggtccaa tttctgggga gcattgagtc ctgatggata ctatgcacga tctgaagatt atgtggatat tgttcaaggg aatagagtag gagtatggaa tgtcccatat atggctaatg tgtacttaat taaaggaaag acactccgat cagagatgaa tgaaaggaac tattttgttc gtgataaact ggatcctgat atggctcttt gccgaaatgc tagagaaatg ggtgtattta tgtacatttc taatagacat gaatttggaa ggctattatc cactgctaat tacaatactt cccattataa caatgacctc tggcagattt ttgaaaatcc tgtggactgg aaggaaaagt atataaaccg tgattattca aagattttca ctgaaaatat agttgaacag ccctgtccag atgtcttttg gttccccata ttttctgaaa aagcctgtga tgaattggta gaagaaatgg aacattacgg caaatggtct gggggaaaac atcatgatag ccgtatatct ggtggttatg aaaatgtccc aactgatgat atccacatga agcaagttga tctggagaat gtatggcttg attttatccg ggagttcatt gcaccagtta cactgaaggt ctttgcaggc tattatacga agggatttgc actactgaat tttgtagtaa aatactcccc tgaacgacag cgttctcttc gtcctcatca tgatgcttct acatttacca taaacattgc acttaataac gtgggagaag actttcaggg aggtggttgc aaatttctaa ggtacaattg ctctattgag tcaccacgaa aaggctggag cttcatgcat cctgggagac tcacacattt gcatgaagga cttcctgtta aaaatggaac aagatacatt gcagtgtcat ttatagatcc ctaagttatt tacttttcat tgaattgaaa tttattttgg gtgaatgact ggcatgaaca cgtctttgaa gttgtggctg agaagatgag aggaatattt aaataacatc aacagaacaa cttcactttg ggccaaacat ttgaaaaact ttttataaaa aattgtttga tatttcttaa tgtctgctct gagccttaaa acacagattg aagaagaaaa gaaagaaaaa acttaaatat ttatttctat gctttgttgc ctctgagaat aatgacaatt tatgaatttg tgtttcaaat tgataaaata tttaggtaca aataacaaga ctaataatat tttcttattt aaaaaaagca tgggaagatt tttatttatc aaaatataga ggaaatgtag acaaaatgga tataaatgaa aattaccatg ttgtaaaacc ttgaaaatca gattctaact gattgtatgc aactaagtat ttctgaacac ctatgcaggt cttatttaca gtgttactaa gggaacacac aaagaattac acaacgtttt cctcaagaaa atggtacaaa acacaaccga ggagcgtata cagttgaaaa cattttgtt ttgattggaa ggcagattat tttatattag tattaaaaat caaaccctat gtttctttca gatgaatctt ccaaagtgga ttatattaag caggtattag atttagaaaa cctttccatt tcttaaagta ttatcaagtg tcaagatcag caagtgtcct taagtcaaat aggtttttt ttgttggtgg ttgtgcttgc tttccttttt tagaaagttc tagaaaatag gaaaacgaaa aatttcattg agatgagtag tgcatttaat tatttttaa aaaacttttt aagtacttga attttatatc aggaaaacaa agttgttgag ccttgcttct tccgttttgc cctttgtctc gctccttatt ctttttggg gggagggtta tttgcttttt tatcttcctg gcataatttc catttattc ttctgagtgt ctatgttaac ttccctctat cccgcttata aaaaaattct ccaacaaaaa tacttgttga cttgatgttt tatcacttct ctaagtaagg ttgaaatatc cttattgtag ctactgtttt taatgtaaag gttaaacttg aaaagaaatt cttaatcacg gtgccaaaat tcattttcta acaccatgtg ttagaaaatt ataaaaaata aaataatttt aaaaaaaaaa aaaaaaaaaa aaa

Figure 23A

MLQNSAVLLVLVISASATHEAEQNDSVSPRKSRVAAQNSAEVVRCLNSALQVGCGAFACLENST
CDTDGMYDICKSFLYSAAKFDTQGKAFVKESLKCIANGVTSKVFLAIRRCSTFQRMIAEVQEECY
SKLNVCSIAKRNPEAITEVVQLPNHFSNRYYNRLVRSLLECDEDTVSTIRDSLMEKIGPNMASLFH
ILQTDHCAQTHPRADFNRRRTNEPQKLKVLLRNLRGEEDSPSHIKRTSHESA

Figure 23B

cagtttgcaa aagccagagg tgcaagaagc agcgactgca gcagcagcag cagcagcggc ggtggcagca gcagcagcag cggcggcagc agcagcagca gcggaggcac cggtggcagc agcagcatca ccagcaacaa caacaaaaaa aaatcctcat caaatcctca cctaagcttt cagtgtatcc agatccacat cttcactcaa gccaggagag ggaaagagga aaggggggca ggaaaaaaaa aaaacccaac aacttagcgg aaacttctca gagaatgctc caaaactcag cagtgcttct ggtgctggtg atcagtgctt ctgcaaccca tgaggcggag cagaatgact ctgtgagccc caggaaatcc cgagtggcgg ctcaaaactc agctgaagtg gttcgttgcc tcaacagtgc tctacaggtc ggctgcgggg ctttgcatg cctggaaaac tccacctgtg acacagatgg gatgtatgac atctgtaaat ccttcttgta cagcgctgct aaatttgaca ctcagggaaa agcattcgtc aaagagagct taaaatgcat cgccaacggg gtcacctcca aggtcttcct cgccattcgg aggtgctcca ctttccaaag gatgattgct gaggtgcagg aagagtgcta cagcaagctg aatgtgtgca gcatcgccaa gcggaaccct gaagccatca ctgaggtcgt ccagctgccc aatcacttct ccaacagata ctataacaga cttgtccgaa gcctgctgga atgtgatgaa gacacagtca gcacaatcag agacagcctg atggagaaaa ttgggcctaa catggccagc ctcttccaca tcctgcagac agaccactgt gcccaaacac acccacgagc tgacttcaac aggagacgca ccaatgagcc gcagaagctg aaagtcctcc tcaggaacct ccgaggtgag gaggactctc cctcccacat caaacgcaca tcccatgaga gtgcataacc agggagaggt tattcacaac ctcaccaaac tagtatcatt ttaggggtgt tgacacacca attttgagtg tactgtgcct ggtttgattt ttttaaagta gttcctattt tctatccccc ttaaagaaaa ttgcatgaaa ctaggcttct gtaatcaata tcccaacatt ctgcaatggc agcattccca ccaacaaaat ccatgtgatc attctgcctc tcctcaggag aaagtaccct cttttaccaa cttcctctgc catgtctttt cccctgctcc cctgagacca cccccaaaca caaaacattc atgtaactct ccagccattg taatttgaag atgtggatcc ctttagaacg gttgccccag tagagttagc tgataaggaa actttattta aatgcatgtc ttaaatgctc ataaagatgt taaatggaat tcgtgttatg aatctgtgct ggccatggac gaatatgaat gtcacatttg aattcttgat ctctaatgag ctagtgtctt atggtcttga tcctccaatg tctaattttc tttccgacac atttaccaaa ttgcttgagc ctggctgtcc aaccagactt tgagcctgca tcttcttgca tctaatgaaa aacaaaaagc taacatcttt acgtactgta actgctcaga gctttaaaag tatctttaac aattgtctta aaaccagaga atcttaaggt ctaactgtgg aatataaata gctgaaaact aatgtactgt acataaattc cagaggactc tgcttaaaca aagcagtata taataacttt attgcatata gatttagttt tgtaacttag ctttattttt cttttcctgg gaatggaata actatctcac ttccagatat ccacataaat gctccttgtg gccttttta taactaaggg ggtagaagta gttttaattc aacatcaaaa cttaagatgg gcctgtatga gacaggaaaa accaacaggt ttatctgaag gaccccaggt aagatgttaa tctcccagcc cacctcaacc cagaggctac tcttgactta gacctatact gaaagatctc tgtcacatcc aactggaaat tccaggaacc aaaaagagca tccctatggg cttggaccac ttacagtgtg ataaggccta ctatacatta ggaagtggta gttctttact cgtccccttt catcggtgcc tggtactctg gcaaatgatg atggggtggg agactttcca ttaaatcaat caggaatgag tcaatcagcc tttaggtctt tagtccgggg gacttggggc tgagagagta taaataaccc tgggctgtcc agccttaata gacttctctt acattttcgt cctgtagcac gctgcctgcc aaagtagtcc tggcagctgg accatctctg taggatcgta aaaaaataga aaaaaagaaa aaaaaaagaa agaaagaggg aaaaagagct ggtggtttga tcatttctgc catgatgttt acaagatggc gaccaccaaa gtcaaacgac taacctatct atgaacaaca gtagtttctc agggtcactg tccttgaacc caacagtccc ttatgagcgt cactgcccac caaaggtcaa tgtcaagaga ggaagagagg gaggaggggt aggactgcag gggccactcc aaactcgctt aggtagaaac tattggtgct cgactctcac taggctaaac tcaagatttg accaaatcga gtgataggga tcctggtggg aggagagagg gcacatctcc agaaaaatga aaagcaatac aactttacca taaagccttt aaaaccagta acgtgctgct caaggaccaa gagcaattgc agcagaccca gcagcagcag cagcagcaca aacattgctg cctttgtccc cacacagcct ctaagcgtgc tgacatcaga ttgttaaggg cattttata ctcagaactg tcccatcccc aggtccccaa acttatggac actgccttag cctcttggaa atcaggtaga ccatattcta agttagactc ttcccctccc tcccacactt cccaccccca ggcaaggctg acttctctga atcagaaaag ctattaaagt ttgtgtgttg tgtccatttt gcaaacccaa ctaagccagg accccaatgc gacaagtagt tcatgagtat tcctagcaaa tttctctctt tcttcagttc agtagatttc ctttttctt ttctttttt ttttttttt ttttggctg tgacctcttc aaaccgtggt acccccccct ttctccccac gatgatatct atatatgtat ctacaataca tatatctaca catacagaaa gaagcagttc tcacatgttg ctagtttttt gcttctcttt cccccaccct actcctcca attcccccct taaacttcca aagcttcgtc ttgtgtttgc tgcagagtga ttcgggggct gacctagacc agtttgcatg attcttctct tgtgatttgg ttgcacttta gacatttttg

Figure 23B (Continued)

tgccattata tttgcattat gtatttataa tttaaatgat atttaggttt ttggctgagt actggaataa acagtgagca tatctggtat atgtcattat ttattgttaa attacatttt ttaagctcca tgtgcatata aaggttatga aacatatcat ggtaatgaca gatgcaagtt attttatttg cttatttttt ataattaaag atgccatagc ataatatgaa gcctttggtg aattccttct aagataaaaa taataataaa gtgttacgtt ttattggttt caaaaaaaaa aaaaaaaaaa a

Figure 24A

MGIGRSEGGRRGALGVLLALGAALLAVGSASEYDYVSFQSDIGPYQSGRFYTKPPQCVDIPADL
RLCHNVGYKKMVLPNLLEHETMAEVKQQASSWVPLLNKNCHAGTQVFLCSLFAPVCLDRPIYPC
RWLCEAVRDSCEPVMQFFGFYWPEMLKCDKFPEGDVCIAMTPPNATEASKPQGTTVCPPCDN
ELKSEAIIEHLCASEFALRMKIKEVKKENGDKKIVPKKKKPLKLGPIKKKDLKKLVLYLKNGADCPC
HQLDNLSHHFLIMGRKVKSQYLLTAIHKWDKKNKEFKNFMKKMKNHECPTFQSVFK

Figure 24B

cctgcagcct ccggagtcag tgccgcgcgc ccgccgcccc gcgccttcct gctcgccgca cctccgggag ccggggcgca cccagcccgc agcgccgcct ccccgcccgc gccgcctccg accgcaggcc gagggccgcc actggccggg gggaccgggc agcagcttgc ggccgcggag ccgggcaacg ctggggactg cgcctttgt ccccggaggt ccctggaagt ttgcggcagg acgcgcgcgg ggaggcggcg gaggcagccc cgacgtcgcg gagaacaggg cgcagagccg gcatgggcat cgggcgcagc gaggggggcc gccgcggggc cctgggcgtg ctgctggcgc tgggcgcggc gcttctggcc gtgggctcgg ccagcgagta cgactacgtg agcttccagt cggacatcgg cccgtaccag agcgggcgct tctacaccaa gccacctcag tgcgtggaca tccccgcgga cctgcggctg tgccacaacg tgggctacaa gaagatggtg ctgcccaacc tgctggagca cgagaccatg gcggaggtga agcagcaggc cagcagctgg gtgcccctgc tcaacaagaa ctgccacgcc gggacccagg tcttcctctg ctcgctcttc gcgcccgtct gcctggaccg gcccatctac ccgtgtcgct ggctctgcga ggccgtgcgc gactcgtgcg agccggtcat gcagttcttc ggcttctact ggcccgagat gcttaagtgt gacaagttcc cggaggggga cgtctgcatc gccatgacgc cgcccaatgc caccgaagcc tccaagcccc aaggcacaac ggtgtgtcct ccctgtgaca acgagttgaa atctgaggcc atcattgaac atctctgtgc cagcgagttt gcactgagga tgaaaataaa agaagtgaaa aaagaaaatg gcgacaagaa gattgtcccc aagaagaaga agcccctgaa gttggggccc atcaagaaga aggacctgaa gaagcttgtg ctgtacctga agaatggggc tgactgtccc tgccaccagc tggacaacct cagccaccac ttcctcatca tgggccgcaa ggtgaagagc cagtacttgc tgacggccat ccacaagtgg gacaagaaaa acaaggagtt caaaaacttc atgaagaaaa tgaaaaacca tgagtgcccc acctttcagt ccgtgtttaa gtgattctcc cgggggcagg gtggggaggg agcctcgggt ggggtgggag cgggggggac agtgcccggg aacccgtggt cacacacacg cactgccctg tcagtagtgg acattgtaat ccagtcggct tgttcttgca gcattcccgc tccctttccc tccatagcca cgctccaaac cccagggtag ccatggccgg gtaaagcaag ggccatttag attaggaagg tttttaagat ccgcaatgtg gagcagcagc cactgcacag gaggaggtga caaaccattt ccaacagcaa cacagccact aaaacacaaa aagggggatt gggcggaaag tgagagccag cagcaaaaac tacattttgc aacttgttgg tgtggatcta ttggctgatc tatgcctttc aactagaaaa ttctaatgat tggcaagtca cgttgttttc aggtccagag tagtttcttt ctgtctgctt taaatggaaa cagactcata ccacacttac aattaaggtc aagcccagaa agtgataagt gcagggagga aaagtgcaag tccattatct aatagtgaca gcaaagggac caggggagag gcattgcctt ctctgcccac agtctttccg tgtgattgtc tttgaatctg aatcagccag tctcagatgc cccaaagttt cggttcctat gagcccgggg catgatctga tccccaagac atgtggaggg gcagcctgtg cctgcctttg tgtcagaaaa aggaaaccac agtgagcctg agagagacgg cgattttcgg gctgagaagg cagtagtttt caaaacacat agttaaaaaa gaaacaaatg aaaaaaattt tagaacagtc cagcaaattg ctagtcaggg tgaattgtga aattgggtga agagcttagg attctaatct catgtttttt ccttttcaca tttttaaaag aacaatgaca aacacccact tatttttcaa ggttttaaaa cagtctacat tgagcatttg aaaggtgtgc tagaacaagg tctcctgatc cgtccgaggc tgcttcccag aggagcagct ctccccaggc atttgccaag ggaggcggat ttccctggta gtgtagctgt gtggctttcc ttcctgaaga gtccgtggtt gccctagaac ctaacacccc ctagcaaaac tcacagagct ttccgttttt ttctttcctg taaagaaaca tttcctttga acttgattgc ctatggatca aagaaattca gaacagcctg cctgttcccc cgcacttttt acatatattt gtttcatttc tgcagatgga aagttgacat gggtgggggtg tccccatcca gcgagagagt ttcaaaagca aaacatctct gcagtttttc ccaagtaccc tgagatactt cccaaagccc ttatgtttaa tcagcgatgt atataagcca gttcacttag acaactttac ccttcttgtc caatgtacag gaagtagttc taaaaaaaat gcatattaat ttcttccccc aaagccggat tcttaattct ctgcaacact ttgaggacat ttatgattgt ccctctgggc caatgcttat acccagtgag gatgctgcag tgaggctgta aagtggcccc ctgcggccct agcctgaccc ggagaaagga tggtagattc tgttaactct tgaagactcc agtatgaaaa tcagcatgcc cgcctagtta cctaccggag agttatcctg ataaattaac ctctcacagt tagtgatcct gtccttttaa caccttttt gtggggttct ctctgacctt tcatcgtaaa gtgctgggga ccttaagtga tttgcctgta attttggatg attaaaaaat gtgtatatat attagctaat tagaaatatt ctacttctct gttgtcaaac tgaaattcag agcaagttcc tgagtgcgtg gatctgggtc ttagttctgg ttgattcact caagagttca gtgctcatac gtatctgctc attttgacaa agtgcctcat gcaaccgggc cctctctctg cggcagagtc cttagtggag gggtttacct ggaacataag tagttaccac agaatacgga agagcaggtg actgtgctgt gcagctctct aaatgggaat tctcaggtag gaagcaacag cttcagaaag agctcaaaat aaattggaaa tgtgaatcgc agctgtgggt tttaccaccg tctgtctcag agtcccagga ccttgagtgt cattagttac

Figure 24B (Continued)

tttattgaag gttttagacc catagcagct ttgtctctgt cacatcagca atttcagaac caaaagggag gctctctgta ggcacagagc tgcactatca cgagcctttg tttttctcca caaagtatct aacaaaacca atgtgcagac tgattggcct ggtcattggt ctccgagaga ggaggtttgc ctgtgatttg cctgtgattt cctaattatc gctagggcca aggtgggatt tgtaaagctt tacaataatc attctggata gagtcctggg aggtccttgg cagaactcag ttaaatcttt gaagaatatt tgtagttatc ttagaagata gcatgggagg tgaggattcc aaaaacattt tatttttaaa atatcctgtg taacacttgg ctcttggtac ctgtgggtta gcatcaagtt ctccccaggg tagaattcaa tcagagctcc agtttgcatt tggatgtgta aattacagta atcccatttc ccaaacctaa aatctgtttt tctcatcaga ctctgagtaa ctggttgctg tgtcataact tcatagatgc aggaggctca ggtgatctgt ttgaggagag caccctaggc agcctgcagg gaataacata ctggccgttc tgacctgttg ccagcagata cacaggacat ggatgaaatt cccgtttcct ctagtttctt cctgtagtac tcctctttta gatcctaagt ctcttacaaa agctttgaat actgtgaaaa tgttttacat tccatttcat ttgtgttgtt tttttaactg cattttacca gatgttttga tgttatcgct tatgttaata gtaattcccg tacgtgttca ttttattttc atgcttttc agccatgtat caatattcac ttgactaaaa tcactcaatt aatcaatgaa aaaaaaaaa

Figure 25A

MADNFSLHDALSGSGNPNPQGWPGAWGNQPAGAGGYPGASYPGAYPGQAPPGAYPGQAPP
GAYHGAPGAYPGAPAPGVYPGPPSGPGAYPSSGQPSAPGAYPATGPYGAPAGPLIVPYNLPLP
GGVVPRMLITILGTVKPNANRIALDFQRGNDVAFHFNPRFNENNRRVIVCNTKLDNNWGREERQ
SVFPFESGKPFKIQVLVEPDHFKVAVNDAHLLQYNHRVKKLNEISKLGISGDIDLTSASYTMI

Figure 25B

ccagccaacg agcggaaaat ggcagacaat ttttcgctcc atgatgcgtt atctgggtct ggaaacccaa accctcaagg atggcctggc gcatgggggа accagcctgc tggggcaggg ggctacccag gggcttccta tcctggggcc taccccgggc aggcaccccc aggggcttat cctggacagg cacctccagg cgcctaccat ggagcacctg gagcttatcc cggagcacct gcacctggag tctacccagg gccacccagc ggccctgggg cctacccatc ttctggacag ccaagtgccc ccggagccta ccctgccact ggcccctatg gcgcccctgc tgggccactg attgtgcctt ataacctgcc tttgcctggg ggagtggtgc ctcgcatgct gataacaatt ctgggcacgg tgaagcccaa tgcaaacaga attgctttag atttccaaag agggaatgat gttgccttcc actttaaccc acgcttcaat gagaacaaca ggagagtcat tgtttgcaat acaaagctgg ataataactg gggaagggaa gaaagacagt cggttttccc atttgaaagt gggaaaccat tcaaaataca agtactggtt gaacctgacc acttcaaggt tgcagtgaat gatgctcact tgttgcagta caatcatcgg gttaaaaaac tcaatgaaat cagcaaactg ggaatttctg gtgacataga cctcaccagt gcttcatata ccatgatata atctgaaagg ggcagattaa aaaaaaaaaa aaagaatcta aaccttacat gtgtaaaggt ttcatgttca ctgtgagtga aaatttttac attcatcaat atccctcttg taagtcatct acttaataaa tattacagtg aaag

Figure 26A

MRTLAILAAILLVALQAQAEPLQARADEVAAAPEQIAADIPEVVVSLAWDESLAPKHPGSRKNMD
CYCRIPACIAGERRYGTCIYQGRLWAFCC

Figure 26B

gaattccctg taagccctgt tacaggggct gcaccccaga tacaacctga cctgtgtcca aggcgggcaa ctcaaccctt agatattgaa
tgggtcccat ggcaccaatg cttaaacacc agcagccctc acaaccacag atcgtgtttt aaggatgagg aggtagttct ctggatgcac
aggcttcaat ccaaatgggc tcatgacgcc gcagcacaca cccagtctgc agcctgaaga gttggagcat tgcattcaca gaaagcatcc
agacatgatc atgggctcag ggatacacct gttctccgat gtgtaccagt gaaggatgga aactcctatg cctcccagaa agcaccactc
aagcttttgc tgaatgcttc tctgaaggcc cacaaggctg agaggctgtg caacaccagc agtaaagtga atgcccagac tcccacctcc
tttcttgggt ggccatctgg aaaggccact cccaccctga tggctaatgc ctcagaccag ttcttggccc agatgatcct agacaattgt
ttaagcttaa actgttcatt ggccaagcaa acaggtgata gtacctctgg ggaaccacat gccgcgtgta catccagatc tcaggagaac
ccaaaaatgt ctgttccaca tagcaacaga agcccaggta gcactcagtc tcacctgggt gttctccaac atcccagctc agccaaatgg
ctttcattag tttttatggt tagaccccag gtcctcggga cactgcttta gaaacacatt ccaaatcctc ctctgtgtgc aggtggcatt cctatcccaa
tctctttgca gggcgtatac tgtgatacgc agccaggctg tcccagaggc cttaaatatt cccttggtgc aggtagttca gcttagccac
agccaatgca tcacagggtc aactgtgtta ggagccattg agaatccata gttggttgct gcctgggcct ggccagggct gaccaaggta
gatgagaggt tcctctgtgg agttctactt taacctcacc ttcccaccaa atttctcaac tgtccttgcc accacaatta tttaatggac
ccaacagaaa gtaaccccgg aaattaggac acctcatccc aaaagacctt taaatagggg aagtccactt gtgcacggct gctccttgct
atagaagacc tgggacagag gactgctgtc tgccctctct ggtcaccctg cctagctaga ggatctgtaa gtactacaaa acttaaactt
tacactgagt tttcatcatt gaagctatgc ctccaatctg acctctgact gtggggccgc cccagaggga cccagcgggt gaatccctgc
taggaacgtc tgtccggacc tctggtgact gctggggacg atggcttcca gctaacttaa tagagaaact caagcagttt ccttctaaat
acacatgtca catgtcctgg ttgacatgtc cagtaagaag actatcacag gtctttggaa cattcttttg agagaaacct atttaggtcc ttggtctgtt
tttcaatcag gttgtttgat ttttgctatt gagttgttgg aattccttat gtattcagat atttgcccct tctgccatgt aggttttgca aatatttttct ctcatttttct
gggttatctt ttcactcggt tgattgtttc ctttgctgtg cagatgcttt agcgttaaat gaagccacac ttgtctattt tccctttttat tgcctgtgcc
tttggtgtca tagccaagaa atcattacct acatcaatgt caaaagcttt atccttctat acacttctag tagtttatgg tttcagttgt tacatttagg
ttttcaattc attctgagtt gatgttccta catggtgtga gataaagatt taaatacata catatataaa atcatgaggt agtgtacact ataaatatac
aattgttaat tgttactcaa gtctaagtag aggtggaaat aataaacttt ctttttttta cttaaaccac tctgtgtcac tgagctgatt tcacctttag
cctgataaaa tcattgtcct ctccaccctg attcctacag gagactactc accccataac ctcaaaaacc tcttcatgag gatggtaagt
cacctgaatc ctgaagtgaa ttactcgcta ttccattgga actcatatag gacaccagaa tctagacctc cagagaacag caggacccat
cttcagaaaa taagaagcat ttgttccctg agcctgttga atcaaagtgc aatttctatt ctttttggaa tgttaaaaag tgaatcataa tatttaagca
ggtgaaccca cgagtaacat agcagggtct ttcttgtcat tattagctcc aacctagcac agacattaaa ggtacagatg tatactagca
tgaaactggg agaacaggag cattcgagca accttgagac caatgggcct ctcttataaa atgcacacct cctctcactg agattgagga
aggtttcttg tctccgagcc ttctcccagt agagctataa atccaggctg gctcctccct ccccacacag ctgctcctgc tctccctcct
ccaggtgacc ccagccatga ggaccctcgc catccttgct gccattctcc tggtggccct gcaggcccag gctgagccac tccaggcaag
agctgatgag gttgctgcag ccccggagca gattgcagcg gacatcccag aagtggttgt ttcccttgca tgggacgaaa gcttggctcc
aaagcatcca ggtgagagag gcaggcatgc agagctgcta agtctagagg gaaggacggg agagaggttc cagagttggg tctcagcagt
ctatgtcact gaggtggctt cacttagaat ctctgggcat tgattttctc atctagaaat tgaacagaga gccaaataaa cctgagaaac
tttatttctc caaagacttg attccaagaa acatctgtga aattcactaa gtttaagata tgaagagaca gactagttat ttctggatct
aaacaagtag acttagttgt aaagagaaca ttttactcta tctacagaag agcttttaaa aactgcagcc aagcctgagg gtaagttcag
gtgtgtgtgt gatggggcag gaatgcaaaa atgagagcaa aggagaatga gtctcaaatt ctgtgtgaca agcactgctc tgcgtgttta
ttcctatcga ctgaggttgt tcgtgctacc ggctgcaatg cagccagcat cacctgtcag ctagcatgtg acttccccga gattcttttt cttacccact
gctaactcca tactcaattt ctcatgctct ccctgtccca ggctcaagga aaaacatgga ctgctattgc agaataccag cgtgcattgc
aggagaacgt cgctatggaa cctgcatcta ccagggaaga ctctgggcat tctgctgctg agcttgcaga aaaagaaaaa tgagctcaaa
atttgctttg agagctacag ggaattgcta ttactcctgt accttctgct caatttcctt tcctcatctc aaataaatgc ctgttacaa gatttctgtg
tttccacctc tttaatgtgt gatatgtgtc tgtgtcaaga cacttgggat acacgtacca aaacgcaaaa tcaaattttt gaacaatata

Figure 27 A

SLWLIAAALVEVRTSADGQAGNEEMVQIDLPIKRYREYELVTPVSTNLEGRYLSHTLSASHKKRS
ARDVSSNPEQLFFNITAFGKDFHLRLKPNTQLVAPGAVVEWHETSLVPGNITDPINNHQPGSATY
RIRKTEPLQTNCAYVGDIVDIPGTSVAISNCDGLAGMIKSDNEEYFIEPLERGKQMEEEKGRIHVV
YKRSAVEQAPIDMSKDFHYRESDLEGLDDLGTVYGNIHQQLNETMRRRRHAGENDYNIEVLLGV
DDSVVRFHGKEHVQNYLLTLMNIVNEIYHDESLGVHINVVLVRMIMLGYAKSISLIERGNPSRSLE
NVCRWASQQQRSDLNHSEHHDHAIFLTRQDFGPAGMQGYAPVTGMCHPVRSCTLNHEDGFSS
AFVVAHETGHVLGMEHDGQGNRCGDETAMGSVMAPLVQAAFHRYHWSRCSGQELKRYIHSYD
CLLDDPFDHDWPKLPELPGINYSMDEQCRFDFGVGYKMCTAFRTFDPCKQLWCSHPDNPYFCK
TKKGPPLDGTECAAGKWCYKGHCMWKNANQQKQDGNWGSWTKFGSCSRTCGTGVRFRTRQ
CNNPMPINGGQDCPGVNFEYQLCNTEECQKHFEDFRAQQCQQRNSHFEYQNTKHHWLPYEH
PDPKKRCHLYCQSKETGDVAYMKQLVHDGTHCSYKDPYSICVRGECVKVGCDKEIGSNKVEDK
CGVCGGDNSHCRTVKGTFTRTPRKLGYLKMFDIPPGARHVLIQEDEASPHILAIKNQATGHYILN
GKGEEAKSRTFIDLGVEWDYNIEDDIESLHTDGPLHDPVIVLIIPQENDTRSSLTYKYIIHEDSVPTI
NSNNVIQEELDTFEWALKSWSQVSKPCGGGFQYTKYGCRRKSDNKMVHRSFCEANKKPKPIRR
MCNIQECTHPLWVAEEWEHCTKTCGSSGYQLRTVRCLQPLLDGTNRSVHSKYCMGDRPESRR
PCNRVPCPAQWKTGPWSECSVTCGEGTEVRQVLCRAGDHCDGEKPESVRACQLPPCNDEPC
LGDKSIFCQMEVLARYCSIPGYNKLCCESCSKRSSTLPPPYLLEAAETHDDVISNPSDLPRSLVM
PTSLVPYHSETPAKKMSLSSISSVGGPNAYAAFRPNSKPDGANLRQRSAQQAGSKTVRLVTVPS
SPPTKRVHLSSASQMAAASFFAASDSIGASSQARTSKKDGKIIDNRRPTRSSTLER

Figure 27B

gtcactttgg ttgatagcag ccgctctggt agaggttagg acttcagctg atggacaagc tggtaatgaa gaaatggtgc aaatagattt
accaataaag agatatagag agtatgagct ggtgactcca gtcagcacaa atctagaagg acgctatctc tcccatactc tttctgcgag
tcacaaaaag aggtcagcga gggacgtgtc ttccaaccct gagcagttgt tctttaacat cacggcattt ggaaaagatt ttcatctgcg
actaaagccc aacactcaac tagtagctcc tggggctgtt gtggagtggc atgagacatc tctggtgcct gggaatataa ccgatcccat
taacaaccat caaccaggaa gtgctacgta tagaatccgg aaaacagagc ctttgcagac taactgtgct tatgttggtg acatcgtgga
cattccagga acctctgttg ccatcagcaa ctgtgatggt ctggctggaa tgataaaaag tgataatgaa gagtatttca ttgaaccctt
ggaaagaggt aaacagatgg aggaagaaaa aggaaggatt catgttgtct acaagagatc agctgtagaa caggctccca tagacatgtc
caaagacttc cactacagag agtcggacct ggaaggcctt gatgatctag gtactgttta tggcaacatc caccagcagc tgaatgaaac
aatgagacgc cgcagacacg cgggagaaaa cgattacaat atcgaggtac tgctgggagt ggatgactct gtggtccgtt tccatggcaa
agagcacgtc caaaactacc tcctgaccct aatgaacatt gtgaatgaaa tttaccatga tgagtcctc ggagtgcata taaatgtggt
cctggtgcgc atgataatgc tgggatatgc aaagtccatc agcctcatag aaaggggaaa cccatccaga agcttggaga atgtgtgtcg
ctgggcgtcc caacagcaaa gatctgatct caaccactct gaacaccatg accatgcaat ttttttaacc aggcaagact ttggacctgc
tggaatgcaa ggatatgctc cagtcaccgg catgtgtcat ccagtgagaa gttgtaccct gaatcatgag gatggttttt catctgcttt
tgtagtagcc catgaaacgg gccatgtgtt gggaatggag catgatggac aaggcaacag gtgtggtgat gagactgcta tgggaagtgt
catggctccc ttggtacaag cagcattcca tcgttaccac tggtcccgat gcagtggtca agaactgaaa agatatatcc attcctatga
ctgtctcctt gatgaccctt ttgatcatga ttggcctaaa ctcccagaac ttcctggaat caattattct atggatgagc aatgtcgttt tgattttggt
gttggctata aaatgtgcac cgcgttccga acctttgacc catgtaaaca gctgtggtgt agccatcctg ataatcccta cttttgtaag
actaaaaagg gacctccact tgatgggact gaatgtgctg ctggaaaatg gtgctataag ggtcattgca tgtggaagaa tgctaatcag
caaaaacaag atggcaattg ggggtcatgg actaaatttg gctcctgttc tcggacatgt ggaactggtg ttcgtttcag aacacgccag
tgcaataatc ccatgcccat caatggtggt caggattgtc ctggtgttaa ttttgagtac cagctttgta acacagaaga atgccaaaaa
cactttgagg acttcagagc acagcagtgt cagcagcgaa actcccactt tgaataccag aataccaaac accactggtt gccatatgaa
catcctgacc ccaagaaaag atgccacctt tactgtcagt ccaaggagac tggagatgtt gcttacatga aacaactggt gcatgatgga
acgcactgtt cttacaaaga tccatatagc atatgtgtgc gaggagagtg tgtgaaagtg ggctgtgata aagaaattgg ttctaataag
gttgaggata agtgtggtgt ctgtggagga gataattccc actgccgaac cgtgaagggg acatttacca gaactcccag gaagcttggg
taccttaaga tgtttgatat acccccctggg gctagacatg tgttaatcca agaagacgag gcttctcctc atattcttgc tattaagaac
caggctacag gccattatat tttaaatggc aaaggggagg aagccaagtc gcggaccttc atagatcttg gtgtggagtg ggattataac
attgaagatg acattgaaag tcttcacacc gatggacctt tacatgatcc tgttattgtt ttgattatac ctcaagaaaa tgatacccgc
tctagcctga catataagta catcatccat gaagactctg tacctacaat caacagcaac aatgtcatcc aggaagaatt agatactttt
gagtgggctt tgaagagctg gtctcaggtt tccaaaccct gtggtggagg tttccagtac actaaatatg gatgccgtag gaaaagtgat
aataaaatgg tccatcgcag cttctgtgag gccaacaaaa agccgaaacc tattagacga atgtgcaata ttcaagagtg tacacatcca
ctctgggtag cagaagaatg ggaacactgc accaaaacct gtggaagttc tggctatcag cttcgcactg tacgctgcct tcagccactc
cttgatggca ccaaccgctc tgtgcacagc aaatactgca tgggtgaccg tcccgagagc cgccggccct gtaacagagt gccctgccct
gcacagtgga aaacaggacc ctggagtgag tgttcagtga cctgcggtga aggaacggag gtgaggcagg tcctctgcag ggctggggac
cactgtgatg gtgaaaagcc tgagtcggtc agagcctgtc aactgcctcc ttgtaatgat gaaccatgtt tgggagacaa gtccatattc
tgtcaaatgg aagtgttggc acgatactgc tccataccag gttataacaa gttatgttgt gagtcctgca gcaagcgcag tagcaccctg
ccaccaccat accttctaga agctgctgaa actcatgatg atgtcatctc taaccctagt gacctcccta gatctctagt gatgcctaca
tctttggttc cttatcattc agagacccct gcaaagaaga tgtctttgag tagcatctct tcagtgggag gtccaaatgc atatgctgct
ttcaggccaa acagtaaacc tgatggtgct aatttacgcc agaggagtgc tcagcaagca ggaagtaaga ctgtgagact ggtcaccgta
ccatcctccc cacccaccaa gagggtccac ctcagttcag cttcacaaat ggctgctgct tccttctttg cagccagtga ttcaataggt

Figure 27B (Continued)

gcttcttctc aggcaagaac ctcaaagaaa gatggaaaga tcattgacaa cagacgtccg acaagatcat ccaccttaga aagatgagaa agtgaaccaa aaaggctaga aaccagagga aaacctggac aacctctctc ttcccatggt gcatatgctt gtttaaagtg gaaatctcta tagatcgtca gctcatttta tctgtaattg gaagaacaga aagtgctggc tcactttcta gttgctttca tcctcctttt gttctgcatt gactcattta ccagaattca ttggaagaaa tcaccaaaga ttattacaaa agaaaaatat gttgctaaga ttgtgttggt cgctctctga agcagaaaag ggactggaac caattgtgca tatcagctga ctttttgttt gttttagaaa agttacagta aaaattaaaa agagatacca atggtttaca ctttaacaag aaattttgga tatggaacaa agaattctta gacttgtatt cctatttatc tatattagaa atattgtatg agcaaatttg cagctgttgt gtaaatactg tatattgcaa aaatcagtat tattttaaga gatgtgttct caaatgattg tttactatat tacatttctg gatgttctag gtgcctgtcg ttgagtattg ccttgtttga cattctatag gttaattttc aaagcagagt attacaaaag agaagttaga attacagcta ctgacaatat aaagggtttt gttgaatcaa caatgtgata cgtaaattat agaaaaagaa aagaaacaca aaagctatag atatacagat atcagcttac ctattgcctt ctatacttat aatttaaagg attggtgtct tagtacactt gtggtcacag ggatcaacga atagtaaata atgaactcgt gcaagacaaa actgaaaccc tctttccagg acctcagtag gcaccgttga ggtgtccttt gtttttgtgt gtgtgttc ttttttaatt ttcgcattgt tgacagatac aaacagttat actcaatgta ctgtaataat cgcaaaggaa aaagttttgg gataacttat ttgtatgttg gtagctgaga aaaatatcat cagtctagaa ttgatatttg agtatagtag agctttgggg ctttgaaggc aggttcaaga aagcatatgt cgatggttga gatatttatt ttccatatgg ttcatgttca aatgttcaca accacaatgc atctgactgc aataatgtgc taataattta tgtcagtagt caccttgctc acagcaaagc cagaaatgct ctctccaggg agtagatgta aagtacttgt acatagaatt cagaactgaa gatatttatt aaaagttgat ttttttttct tgatagtatt tttatgtact aaatatttac actaatatca attacatatt ttggtaaact agagagacat aattagagat gcatgctttg ttctgtgcat agagaccttt aagcaaacta ctacagccaa ctcaaaagct aaaactgaac aaatttgatg ttatgcaaac atcttgcatt tttagtagtt gatattaagt tgatgacttg tttcccttca aggaaacatt aaattgtatg gactcagcta gctgttcaat gaaattgtga attagaaaca ttttaaaag tttttgaaag agataagtgc atcatgaatt acatgtacat gagaggagat agtgatatca gcataatgat tttgaggtca gtacctgagc tgtctaaaaa tatattatac aaactaaaat gtagatgaat taacctctca aagcacagaa tgtgcaagaa cttttgcatt ttaatcgttg taaactaaca gcttaaacta ttgactctat acctctaaag aattgctgct actttgtgca agaactttga aggtcaaatt aggcaaattc cagatagtaa aacaatccct aagccttaag tctttttttt ttcctaaaaa ttcccataga ataaaattct ctctagttta cttgtgtgtg catacatctc atccacaggg gaagataaag atggtcacac aaacagtttc cataaagatg tacatattca ttatacttct gacctttggg ctttcttttc tactaagcta aaaattcctt tttatcaaag tgtacactac tgatgctgtt tgttgtactg agagcacgta ccaataaaaa tgttaacaaa atat

Figure 28

tttttttttcagattgaaatcactttaatagcataacaacattttcagaccaggagtcacagatgaagaaaacattttgtcttccatttgcacaattctggtgaggt
gtgtggttgcactggacaatcttacagacacatttttcacattgagaacttaataaatagatacatacaatgtcaaactccacagacaatgagttatgagtgt
gattgttttcttattctgcctcctctgggttgggaggttgcttcccgttgggctgatggcggctgggtcctctaggaggggtactcatactcctcggcactgcgac
ggccaaaatccatccagcccatgtagtcccggtcacttatcctgtggctggggtccaggttctgcaggttcttaacgatggacattcgtccagaaggagctt
tccgggcctgctggatgtatcttgccagcagggcgcccaggtgcgctcgggactcgccatccgttctctgcgataccctcagctgcctacggggcgcctc
ctctgcccgctgcagcccggagcccgcgggatctgcgggaggcaccggctgcgtcagggcgccagccgccagtaccgncatcagcacgcacagg
cacacgccgctgttcat

Figure 29A

MSVKGMAIALAVILCATVVQGFPMFKRGRCLCIGPGVKAVKVADIEKASIMYPSNNCDKIEVIITLK
ENKGQRCLNPKSKQARLIIKKVERKNF

Figure 29B

ctccttccaa gaagagcagc aaagctgaag tagcagcaac agcaccagca gcaacagcaa aaaacaaaca tgagtgtgaa
gggcatggct atagccttgg ctgtgatatt gtgtgctaca gttgttcaag gcttccccat gttcaaaaga ggacgctgtc tttgcatagg
ccctggggta aaagcagtga aagtggcaga tattgagaaa gcctccataa tgtacccaag taacaactgt gacaaaatag aagtgattat
taccctgaaa gaaaataaag gacaacgatg cctaaatccc aaatcgaagc aagcaaggct tataatcaaa aaagttgaaa gaaagaattt
ttaaaaatat caaaacatat gaagtcctgg aaaagggcat ctgaaaaacc tagaacaagt ttaactgtga ctactgaaat gacaagaatt
ctacagtagg aaactgagac ttttctatgg ttttgtgact ttcaactttt gtacagttat gtgaaggatg aaaggtgggt gaaaggacca
aaaacagaaa tacagtcttc ctgaatgaat gacaatcaga attccactgc ccaaaggagt ccagcaatta aatggatttc taggaaaagc
taccttaaga aaggctggtt accatcggag tttacaaagt gctttcacgt tcttacttgt tgtattatac attcatgcat ttctaggcta gagaaccttc
tagatttgat gcttacaact attctgttgt gactatgaga acatttctgt ctctagaagt tatctgtctg tattgatctt tatgctatat tactatctgt
ggttacagtg gagacattga cattattact ggagtcaagc ccttataagt caaaagcatc tatgtgtcgt aaagcattcc tcaaacattt
tttcatgcaa atacacaytt ctttccccaa atatcatgta gcacatcaat atgtagggaa acattcttat gcatcatttg gtttgtttta taaccaattc
attaaatgta attcataaaa tgtactatga aaaaaattat acgctatggg atactggcaa cagtgcacat atttcataac caaattagca
gcaccggtct taatttgatg tttttcaact tttattcatt gagatgtttt gaagcaatta ggatatgtgt gtttactgta ctttttgttt tgatccgttt
gtataaatga tagcaatatc ttggacacat ttgaaataca aaatgttttt gtctaccaaa gaaaaatgtt gaaaaataag caaatgtata
cctagcaatc acttttactt tttgtaattc tgtctcttag aaaaatacat aatctaatca aaaaaaaaaa aaaaaaaaaa a

Figure 30 A

MTRLTVLALLAGLLASSRAGSSPLLDIVGGRKARPRQFPFLASIQNQGRHFCGGALIHARFVMTA
ASCFQSQNPGVSTVVLGAYDLRRRERQSRQTFSISSMSENGYDPQQNLNDLMLLQLDREANLT
SSVTILPLPLQNATVEAGTRCQVAGWGSQRSGGRLSRFPRFVNVTVTPEDQCRPNNVCTGVLT
RRGGICNGDGGTPLVCEGLAHGVASFSLGPCGRGPDFFTRVALFRDWIDGVLNNPGPGPA"

Figure 30B

ggatccactg gttcctgaca ccctcacctg cccctggggg tgtggccatc ttctagagag ggaaactgag gatcagtgca gaatgtaggg ggagcccagg ctggcccagg gagcagttgg cggtggaggc cttgggcaat ttcccgtgtt cccactgagt ggggctgtcc ctgggcctgg gcggggacgc caccaactgc caaggcctgt gtataagggc agccgccgcc ttagccacag acctgccccg ccatgacccg gctgacagtc ctggccctgc tggctggtct gctggcatcc tcgagggccg gtgagtgcct ctctgtgccg gtggtccccc atctgtgcta gggcccggct gccagggcag aactcagact taaagcacag agaaggcaag cggcttggcc tgggtcacac agccagcccg gcctggacga tcccgcgaaa ggcgtgaggg cggacggtgt gcgggactca ggggcccccc gtcctcttag ggagtgggac gatgggggag ggtgggtccc cccgcagccc cactgggtgg atagagctga ggctgcagct tcacacgccc tcccggccac tgtgtggatt cttggggatc tcagagctgt ctcccccccga cccaggctcc agcccccttt tggacatcgt tggcggccgg aaggcgaggc cccgccagtt cccgttcctg gcctccattc agaatcaagg caggcacttc tgcgggggtg ccctgatcca tgcccgcttc gtgatgaccg cggccagctg cttccaaagc cagtgagggg tcctggggag ggggcctagg gggcattggg gctcagagaa ggggcttggg gggcttaggc attcagtggg ggtgcttggt aggtgaggag gggaggggat tgcaaaagga ggggctcagg gaaaggaggg ggcttggaga gggaaatggg gactgagttg aggagggacc caaggatatt ggggggctca gatggaggag gcccagagaa gggaaggggg tcagatggag gaggcccaga gaaaggaaga ggctcagatg gaggaggtgc agtgaaggaa agggggtcag atgggggagg cccagagaag ggaaggggct cagatggagg agggggccca gagaaaggaa ggggctcaga tggaggaggt gcagagaagg gaagggggtc agatgggggg aggcccagag aagggaaggg gctcagatgg aggaggtgca gagaagggaa ggggctcaga tggaggaggt gcagagaaga gaagggcctc agatggagga ggtgcagaga agagaagggc ctcagatgga ggaggtgcag agaagggaag ggcctcagat ggaggaggtg cggagaaggg aaggggtca gatggaggag gtgcagagaa gggaaggggg tcagatgggg gaggcccagg gaagggaagg ggctcagatg gaggaggggc agagaaggga agggggtcag atgggggagg cccagggaag ggaaggggct cagatggggg aggcgcagag aagggaaggg ggtcagatgg aggaggtgca gagaagggaa gggggtcaga tgggggaggc ccagataagg gaatggggtc agatggggga ggtgcagaga agggaagggg gtcagatggg ggaggcccag ataagggaag gggctcagat ggaggaggtg cagagaaggg gaggggtca gatggaggag gctcagagaa gggaagggac tcagatggag gaggggcgc agagaagaga aggggctcag atggaggagg aggcgcagag aagggaaggg gctcaagatg ggaaggggc cctggaaagt ctcggctctg cttctgtaaa agcgggggag ttttcagggt gaaggattgc agtctgcagg ctgggatccc ccctaatttg caagccggct tgctctgtgc ccaggcccca gcctggtgtc ctccctctgc cctttcctcc gctactctca ggaacccccg ggttagcacc gtggtgctgg gtgcctatga cctgaggcgg cgggagaggc agtcccgcca gacgttttcc atcagcagca tgagcgagaa tggctacgac ccccagcaga acctgaacga cctgatgctg cttcaggtga gaggatggtg ccacctgtga tcccagcacc tcgggaggcc gacgttagcc agggaaacaa gtccaaactt ggtctctaca aaaaaataca aaaattagcc gggagtggtg gcgcgcacct gtggcccctg tgcttcagga ggccgaggcg gaaggacggc ttgaggtcag gagttcgaga ccagcctggg caacatggcc aaactcagtc tctacaaaaa tatatatgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtatctt gccgggtgag gtggctcatg cctgtaatcc cagcattttg ggaggccgag gtgggcggat cacgaggtca ggagattgag accagcctgg ccaacatggt gaaaccccat ctctactaaa aatacaaaaa ttagccaggc atggcagcgg gcgcctctag tcccagctac tcaggaggct gaggcaggag aatcgcttga acccgggagg cggagcttgc agtgagccga gatcgcgccc ctgcactcca gcctgggtaa cagagccaga ccctatctca aaaaaaactt ccaaaaacaa tacagcaaca catacagatg taccacggtt cgcgtatgga gcctcctgtt ggtggagact gacgtcgttt tcaaatgctt ttgctatgac agaatcatgt gaatgttttt catgtttggt ttttttcttt gagaaaatga taaaattatc tcaaaaatat cattaaaaaa tttaaaaaag tagagacggg ggtttcacct tgttggccag tttggtctcg aactcctggc ctcaagtgat ccacccacct tggcctcgca acgtgctggg aatacaggcg tgagccaccg cacccggccc ctgccgggaa ttaaacgcaa accacttaca gactacagtt aatgtcgctg acacttctgc tcccaggggt ccccatgagg ctccagtccc cagggccacc ctcccctgac tccatttcct tccccagctg gaccgtgagg ccaacctcac cagcagcgtg acgatactgc cactgcctct gcagaacgcc acggtggaag ccggcaccag atgccaggtg gccggctggg ggagccagcg cagtgggggg cgtctctccc gttttcccag gtttgtcaac gtgactgtga cccccgagga ccagtgtcgc cccaacaacg tgtgcaccgg tgtgctcacc cgccgcggtg gcatctgcaa tgtgagtgct

Figure 30B (Continued)

ccctgtggcg ggaggagggg tcctgagagg tactgagctc tccgtggcag gagaaagcaa gtgcaggctg agggcggcac agcaggggggg ccccaggatt gagcattttc acggtaggag aaacagtatc ttttttttt tttttgagac agagtctcgc tctgtcgccc aggctggagt gtagtggcgt gatctcggcg gctcactgca acctccgcct cctgggttca agcgattctc ctgcctcagc ctcctaagta gctgggatta caggcatgcg ccaccacgcc cggctaattt tgtatttta gtagagacag ggtttctcca tgtgggtcag gctggtctcg aactcctgac ctcatgatcg acccaccttg gcctcccaaa gtgttaggat aacaggcatg agccaccgtg cctggctgag aaacagtagc tatcaaacgc cggctgtgag ccacgtctgt gctgggggtt ggggacccag caggcatggt agagccggtc actgagggac tcaggcgtgt gattgccagg ggaggggcac ctggcccagc ctggaggtgc caggaagctc cagaaagcaa ctgatcccaa agtccactag cagttaacca gggcagagaa agagaagagc catgcaaagg ccctggggct ggatcaggac ttgtaggttc caggggcagc aagaggcctc tgcagttctg gggtggcgtg ggagccaggc cctgggacgc cctgacacag ctgctgcctg cccaggggga cgggggcacc cccctcgtct gcgagggcct ggcccacggc gtggcctcct tttccctggg gccctgtggc cgaggccctg acttcttcac ccgagtggcg ctcttccgag actggatcga tggtgttctc aacaacccgg gaccggggcc agcctagggg ggcctgtgac ctcccatgga gcccagcccc cgccctccac acctccggcg ctccgcaccc acctcccacg gccccgcccc tgcccccgct ccggccagag gggccctggc tgtaataaag aagccgatct ctcctctgct cctggtttct gttcattggt gggggagggg gctgtgggga cgcgtgagtg gcaccttcac cggccttagg ggcacccacc gcaggtgcac tgcctgtgca gatgtcagat gttcagagat tccctcaaag cccggggaag caggggctgg tgttatctgc acccgacagc ggggtgttgg ggggaggccc aggttcagag aggttgggtg gctgcccaga ggtcacacag tgaatgccgc ccagcacttt gggaggccga ggtgggcgga tcacctgagg tcaggagttc aagaccagcc cggccaacct ggtgaaaccc catctctata aaaatacaaa aattagccgg gcatgatggc gggcgcctgt aatcccagtt acttgggagg ctgaggcagg agaatcacct gaacccggga ggcggaggtt gcagcgaacc gagatggcgc cactgcactc cagcctgggc gacagcgaga ctccagctca aaaaaaaaca aaaaccacgg gagaaaacgg ggaacattct cctcttggat cc

Figure 31A

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAAHKWSLP
EMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSKKKETESAIYIFISD
TGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMT
WSYPDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIY
DKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYLTRGYSLIIKD
VTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQP
TIKWFWHPCNHNHSEARCDFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKMASTLVVADS
RISGIYICIASNKVGTVGRNISFYITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRT
VNNRTMHYSISKQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRGEHCN
KKAVFSRISKFKSTRNDCTTQSNVKH

Figure 31B

gcggacactc ctctcggctc ctccccggca gcggcggcgg ctcggagcgg gctccggggc tcgggtgcag cggccagcgg gcctggcggc
gaggattacc cggggaagtg gttgtctcct ggctggagcc gcgagacggg cgctcagggc gcggggccgg cggcggcgaa
cgagaggacg gactctggcg gccgggtcgt tggccggggg agcgcgggca ccgggcgagc aggccgcgtc gcgctcacca tggtcagcta
ctgggacacc ggggtcctgc tgtgcgcgct gctcagctgt ctgcttctca caggatctag ttcaggttca aaattaaaag atcctgaact
gagtttaaaa ggcacccagc acatcatgca agcaggccag acactgcatc tccaatgcag gggggaagca gcccataaat ggtctttgcc
tgaaatggtg agtaaggaaa gcgaaaggct gagcataact aaatctgcct gtggaagaaa tggcaaacaa ttctgcagta ctttaacctt
gaacacagct caagcaaacc acactggctt ctacagctgc aaatatctag ctgtacctac ttcaaagaag aaggaaacag aatctgcaat
ctatatattt attagtgata caggtagacc tttcgtagag atgtacagtg aaatccccga aattatacac atgactgaag gaagggagct
cgtcattccc tgccgggtta cgtcacctaa catcactgtt actttaaaaa agtttccact tgacactttg atccctgatg gaaaacgcat
aatctgggac agtagaaagg gcttcatcat atcaaatgca acgtacaaag aaatagggct tctgacctgt gaagcaacag tcaatgggca
tttgtataag acaaactatc tcacacatcg acaaaccaat acaatcatag atgtccaaat aagcacacca cgcccagtca aattacttag
aggccatact cttgtcctca attgtactgc taccactccc ttgaacacga gagttcaaat gacctggagt taccctgatg aaaaaaataa
gagagcttcc gtaaggcgac gaattgacca aagcaattcc catgccaaca tattctacag tgttcttact attgacaaaa tgcagaacaa
agacaaagga ctttatactt gtcgtgtaag gagtggacca tcattcaaat ctgttaacac ctcagtgcat atatatgata aagcattcat
cactgtgaaa catcgaaaac agcaggtgct tgaaaccgta gctggcaagc ggtcttaccg gctctctatg aaagtgaagg catttccctc
gccggaagtt gtatggttaa aagatgggtt acctgcgact gagaaatctg ctcgctattt gactcgtggc tactcgttaa ttatcaagga
cgtaactgaa gaggatgcag ggaattatac aatcttgctg agcataaaac agtcaaatgt gtttaaaaac ctcactgcca ctctaattgt
caatgtgaaa ccccagattt acgaaaaggc cgtgtcatcg tttccagacc cggctctcta cccactgggc agcagacaaa tcctgacttg
taccgcatat ggtatccctc aacctacaat caagtggttc tggcaccccc gtaaccataa tcattccgaa gcaaggtgtg acttttgttc
caataatgaa gagtccttta tcctggatgc tgacagcaac atgggaaaca gaattgagag catcactcag cgcatggcaa taatagaagg
aaagaataag atggctagca ccttggttgt ggctgactct agaatttctg gaatctacat ttgcatagct tccaataaag ttgggactgt
gggaagaaac ataagctttt atatcacaga tgtgccaaat gggtttcatg ttaacttgga aaaaatgccg acggaaggag aggacctgaa
actgtcttgc acagttaaca agttcttata cagagacgtt acttggattt tactgcggac agttaataac agaacaatgc actacagtat
tagcaagcaa aaaatggcca tcactaagga gcactccatc actcttaatc ttaccatcat gaatgtttcc ctgcaagatt caggcaccta
tgcctgcaga gccaggaatg tatacacagg ggaagaaatc ctccagaaga aagaaattac aatcagaggt gagcactgca acaaaaaggc
tgttttctct cggatctcca aatttaaaag cacaaggaat gattgtacca cacaaagtaa tgtaaaacat taaaggactc attaaaaagt
aacagttgtc tcatatcatc ttgatttatt gtcactgttg ctaactttca ggctcggagg agatgctcct cccaaaatga gttcggagat
gatagcagta ataatgagac ccccgggctc cagctctggg ccccccattc aggccgaggg ggctgctccg gggggccgac ttggtgcacg
tttggatttg gaggatccct gcactgcctt ctctgtgttt gttgctcttg ctgttttctc ctgcctgata aacaacaact tgggatgatc ctttccattt
tgatgccaac ctcttttat ttttaagcgg cgccctatag t

Figure 32A

MSDSVILRSIKKFGEENDGFESDKSYNNDKKSRLQDEKKGDGVRVGFFQLFRFSSSTDIWLMFV
GSLCAFLHGIAQPGVLLIFGTMTDVFIDYDVELQELQIPGKACVNNTIVWTNSSLNQNMTNGTRC
GLLNIESEMIKFASYYAGIAVAVLITGYIQICFWVIAAARQIQKMRKFYFRRIMRMEIGWFDCNSVG
ELNTRFSDDINKINDAIADQMALFIQRMTSTICGFLLGFFRGWKLTLVIISVSPLIGIGAATIGLSVSK
FTDYELKAYAKAGVVADEVISSMRTVAAFGGEKREVERYEKNLVFAQRWGIRKGIVMGFFTGFV
WCLIFLCYAVAFWYGSTLVLDEGEYTPGTLVQIFLSVIVGALNLGNASPCLEAFATGRAAATSIFE
TIDRKPIIDCMSEDGYKLDRIKGEIEFHNVTFHYPSRPEVKILNDLNMVIKPGEMTALVGPSGAGKS
TALQLIQRFYDPCEGMVTVDGHDIRSLNIQWLRDQIGIVEQEPVLFSTTIAENIRYGREDATMEDIV
QAAKEANAYNFIMDLPQQFDTLVGEGGGQMSGGQKQRVAIARALIRNPKILLLDMATSALDNES
EAMVQEVLSKIQHGHTIISVAHRLSTVRAADTIIGFEHGTAVERGTHEELLERKGVYFTLVTLQSQ
GNQALNEEDIKDATEDDMLARTFSRGSYQDSLRASIRQRSKSQLSYLVHEPPLAVVDHKSTYEE
DRKDKDIPVQEEVEPAPVRRILKFSAPEWPYMLVGSVGAAVNGTVTPLYAFLFSQILGTFSIPDKE
EQRSQINGVCLLFVAMGCVSLFTQFLQGYAFAKSGELLTKRLRKFGFRAMLGQDIAWFDDLRNS
PGALTTRLATDASQVQGAAGSQIGMIVNSFTNVTVAMIIAFSFSWKLSLVILCFFPFLALSGATQTR
MLTGFASRDKQALEMVGQITNEALSNIRTVAGIGKERRFIEALETELEKPFKTAIQKANIYGFCFAF
AQCIMFIANSASYRYGGYLISNEGLHFSYVFRVISAVVLSATALGRAFSYTPSYAKAKISAARFFQL
LDRQPPISVYNTAGEKWDNFQGKIDFVDCKFTYPSRPDSQVLNGLSVSISPGQTLAFVGSSGCG
KSTSIQLLERFYDPDQGKVMIDGHDSKKVNVQFLRSNIGIVSQEPVLFACSIMDNIKYGDNTKEIP
MERVIAAAKQAQLHDFVMSLPEKYETNVGSQGSQLSRGEKQRIAIARAIVRDPKILLLDEATSALD
TESEKTVQVALDKAREGRTCIVIAHRLSTIQNADIIAVMAQGVVIEKGTHEELMAQKGAYYKLVTT
GSPIS

Figure 32B

gaatgatgaa aaccgaggtt ggaaaaggtt gtgaaacctt ttaactctcc acagtggagt ccattatttc ctctggcttc ctcaaattca
tattcacagg gtcgttggct gtgggttgca attaccatgt ctgactcagt aattcttcga agtataaaga aatttggaga ggagaatgat
ggttttgagt cagataaatc atataataat gataagaaat caaggttaca agatgagaag aaaggtgatg gcgttagagt tggcttcttt
caattgtttc ggttttcttc atcaactgac atttggctga tgtttgtggg aagtttgtgt gcatttctcc atggaatagc ccagccaggc gtgctactca
tttttggcac aatgacagat gtttttattg actacgacgt tgagttacaa gaactccaga ttccaggaaa agcatgtgtg aataacacca
ttgtatggac taacagttcc ctcaaccaga acatgacaaa tggaacacgt tgtgggttgc tgaacatcga gagcgaaatg atcaaatttg
ccagttacta tgctggaatt gctgtcgcag tacttatcac aggatatatt caaatatgct tttgggtcat tgccgcagct cgtcagatac
agaaaatgag aaaattttac tttaggagaa taatgagaat ggaaataggg tggtttgact gcaattcagt gggggagctg aatacaagat
tctctgatga tattaataaa atcaatgatg ccatagctga ccaaatggcc cttttcattc agcgcatgac ctcgaccatc tgtggtttcc tgttgggatt
tttcaggggt tggaaactga ccttggttat tatttctgtc agccctctca ttgggattgg agcagccacc attggtctga gtgtgtccaa gtttacggac
tatgagctga aggcctatgc caaagcaggg gtggtggctg atgaagtcat ttcatcaatg agaacagtgg ctgcttttgg tggtgagaaa
agagaggttg aaaggtatga gaaaaatctt gtgttcgccc agcgttgggg aattagaaaa ggaatagtga tgggattctt tactggattc
gtgtggtgtc tcatcttttt gtgttatgca gtggccttct ggtacggctc cacacttgtc ctggatgaag gagaatatac accaggaacc
cttgtccaga ttttcctcag tgtcatagta ggagctttaa atcttggcaa tgcctctcct tgtttggaag cctttgcaac tggacgtgca
gcagccacca gcatttttga gacaatagac aggaaaccca tcattgactg catgtcagaa gatggttaca agttggatcg aatcaagggt
gaaattgaat tccataatgt gaccttccat tatccttcca gaccagaggt gaagattcta aatgacctca acatggtcat taaaccaggg
gaaatgacag ctctggtagg acccagtgga gctggaaaaa gtacagcact gcaactcatt cagcgattct atgacccctg tgaaggaatg
gtgaccgtgg atggccatga cattcgctct cttaacattc agtggcttag agatcagatt gggatagtgg agcaagagcc agttctgttc
tctaccacca ttgcagaaaa tattcgctat ggcagagaag atgcaacaat ggaagacata gtccaagctg ccaaggaggc caatgcctac
aacttcatca tggacctgcc acagcaattt gacacccttg ttggagaagg aggaggccag atgagtggtg gccagaaaca aagggtagct
atcgccagag ccctcatccg aaatcccaag attctgcttt tggacatggc cacctcagct ctggacaatg agagtgaagc catggtgcaa
gaagtgctga gtaagattca gcatgggcac acaatcattt cagttgctca tcgcttgtct acggtcagag ctgcagatac catcattggt
tttgaacatg gcactgcagt ggaaagaggg acccatgaag aattactgga aaggaaaggt gtttacttca ctctagtgac tttgcaaagc
cagggaaatc aagctcttaa tgaagaggac ataaaggatg caactgaaga tgacatgctt gcgaggacct ttagcagagg gagctaccag
gatagtttaa gggcttccat ccggcaacgc tccaagtctc agctttctta cctggtgcac gaacctccat tagctgttgt agatcataag
tctacctatg aagaagatag aaaggacaag gacattcctg tgcaggaaga agttgaacct gccccagtta ggaggattct gaaattcagt
gctccagaat ggccctacat gctggtaggg tctgtgggtg cagctgtgaa cgggacagtc acacccttgt atgccttttt attcagccag
attcttggga cttttcaat tcctgataaa gaggaacaaa ggtcacagat caatggtgtg tgcctacttt ttgtagcaat gggctgtgta tctcttttca
cccaatttct acagggatat gcctttgcta aatctgggga gctcctaaca aaaaggctac gtaaatttgg tttcagggca atgctggggc
aagatattgc ctggtttgat gacctcagaa atagccctgg agcattgaca acaagacttg ctacagatgc ttcccaagtt caaggggctg
ccggctctca gatcgggatg atagtcaatt ccttcactaa cgtcactgtg gccatgatca ttgccttctc ctttagctgg aagctgagcc tggtcatctt
gtgcttcttc cccttcttgg ctttatcagg agccacacag accaggatgt tgacaggatt tgcctctcga gataagcagg ccctggagat
ggtgggacag attacaaatg aagccctcag taacatccgc actgttgctg gaattggaaa ggagaggcgg ttcattgaag cacttgagac
tgagctggag aagcccttca agacagccat tcagaaagcc aatatttacg gattctgctt tgcctttgcc cagtgcatca tgtttattgc
gaattctgct tcctacagat atggaggtta cttaatctcc aatgaggggc tccatttcag ctatgtgttc agggtgatct ctgcagttgt actgagtgca
acagctcttg gaagagcctt ctcttacacc ccaagttatg caaaagctaa aatatcagct gcacgctttt ttcaactgct ggaccgacaa
cccccaatca gtgtatacaa tactgcaggt gaaaaatggg acaacttcca ggggaagatt gattttgttg attgtaaatt tacatatcct
tctcgacctg actcgcaagt tctgaatggt ctctcagtgt cgattagtcc agggcagaca ctggcgtttg ttgggagcag tggatgtggc
aaaagcacta gcattcagct gttggaacgt ttctatgatc ctgatcaagg gaaggtgatg atagatggtc atgacagcaa aaaagtaaat

Figure 32B (Continued)

gtccagttcc tccgctcaaa cattggaatt gtttcccagg aaccagtgtt gtttgcctgt agcataatgg acaatatcaa gtatggagac aacaccaaag aaattcccat ggaaagagtc atagcagctg caaaacaggc tcagctgcat gattttgtca tgtcactccc agagaaatat gaaactaacg ttgggtccca ggggtctcaa ctctctagag gggagaaaca acgcattgct attgctcggg ccattgtacg agatcctaaa atcttgctac tagatgaagc cacttctgcc ttagacacag aaagtgaaaa gacggtgcag gttgctctag acaaagccag agagggtcgg acctgcattg tcattgccca tcgcttgtcc accatccaga acgcggatat cattgctgtc atggcacagg gggtggtgat tgaaaagggg acccatgaag aactgatggc ccaaaaagga gcctactaca aactagtcac cactggatcc cccatcagtt gacccaatgc aagaatctca gacacacatg acgcaccagt tacaggggtt gtttttaaag aaaaaaacaa tcccagcacg agggattgct gggattgttt tttctttaaa gaagaatntn nntattttac ttttacnnnc nttttcctac atcggaatcc aanctaattt ctaatggcct tccataataa ttctgcttta gatgtgtata cagaaaatga aagaaactag ggtccatgtg agggaaaacc caatgtcaag tggcagctca gccaccactc agtgcttctc tgtgcaggag ccagtcctga ttaatatgtg ggaattagtg agacatcagg gagtaagtga cactttgaac tcctcaagga cagagaactg tctttcattt ttgaaccctc ggtgtacaca gaggcgggtc tgtaacaggc aatcaacaaa cgtttcttga gctagaccaa ggtcagattt gaaaagaaca gaaggactga agaccagctg tgtttcttaa ctaaatttgt ctttcaagtg aaaccagctt ccttcatctc taaggctaag gatagggaaa gggtgggatg ctctcangct gagggaggca naaagggaaa gtattancat gagctttcca nttagggctg ttgatttatg ctttaacttc anantgagtg tagggtggtg anncta

Figure 33A

MDTQTHSLPITHTQLHSNSQPQSRTCTRHCQTFSQSCRQSHRGSRSQSSSQSPASHRNPTGA
HSSSGHQSQSPNTSPPPKRHKKTMNSHHSPMRPTILHCRCPKNRKNLEGKLKKKKMAKRIQQV
YKTKTRSSGWKSN

Figure 33B

agactcagct taatctgacc caagggctcc taccctgaac cagtagctgg gactatcccc agggtacccc tgagagctgc cccagcctgg ggtgagggta aggggtaggg ggctttgtct tggctgagcc acatctctca cacccctgtg gcctgggcat cataatcagc cccaactata taaccaggtg ggcctgccag ggcctctgta aagctaggcc tgctgggaga ggatgaggag gaggccctgc cctcaaacgt ggcctcctat ggacacccag actcacagcc ttcctatcac ccacactcag ctccatagca actctcagcc ccaaagccgc acctgcaccc gccattgcca aaccttcagc cagagttgca gacagagcca tcgtggcagc cggagccaga gctccagcca gagcccggcc agccaccgca acccaactgg agcccacagc tcatccggcc accagagcca gagtcccaac actagtccac caccaaagcg ccacaaaaag actatgaact cccaccactc tcccatgcgg cccaccatcc tgcactgccg ctgccccaag aacagaaaga acttggaagg caagctgaaa aagaaaaaaa tggccaagag gatccagcag gtgtacaaaa ccaagacgcg gagctcaggt acccttaag gaggtgggga agggccaccg agccacagat gatggagagc agaccttggg ggcagtgaga ggaaggctgc agccaggtca caaaggaacc acaggcaaga aggaagaggg agaagagaaa caatggcagt tggctagctg aatgtatgat acgttgacgg aaagtcttct ttgaaattgg atgggttgat taggaggatg gaaagatgga cagatagcag ataagctaga tgaaagcatg aatggagttg agaggttggg ttgatgactg ggtgggtaaa caataaatag gttatagaaa ggatagttgg aagaatgcat tggctgaatg ataggaagtt tggatacgat tagctggatg gatggataaa tggatgaatg cactggctgg ctagttattt ggttggttag gtagatgatc agtttgaaga ttgtggttgg tggatgaatt ggttagaaat agagttaaat agttgtagaa gttttgatgg gttggtttga ttggttaaat attatcttaa tagagtaata tagagtaatt gaataaacag agagaagaat agatatctag actaatggga tagaatggga aagaaatgtt gaataaatga atggaatgag tgaactaatg aatgggtgga tgacaaatgg aagggataaa tggatggata cctggattca cataggtcaa aaggacactg acggtagtct aaactctatc tatgtcccat atcaatcaca aatgagtagt tgtaagacct tacaggaggt caaggaggtc actgacttca tgaagtgctc agctattaaa ggttcctttc ccactcttat cccttaggat ggaaatccaa ctaatgagac cgcactcctt ggcttgttcc tgcgtgtttc acccaaagga gaaaatgcta ggatgaagtc aatcttcttg caggaacatg ttactatggt gatttctacg caacactaat taaagcttgt acctggaaga ctatccctga gtagtcattt tgatttcact aataaaggtg ttatgtgttt tgggggcctg cacaggggca gaaatgaatg ggggtaggat gccaagaagc ctgcag

Figure 34A

MGKSESQMDITDINTPKPKKKQRWTRLEISLSVLVLLLTIIAVRMIALYATYDDGICKSSDCIKSAAR
LIQNMDATTEPCRDFFKYACGGWLKRNVIPETSSRYGNFDILRDELEVVLKDVLQEPKTEDIVAV
QKAKALYRSCINESAIDSRGGEPLLKLLPDIYGWPVATENWEQKYGASWTAEKAIAQLNSKYGKK
VLINLFVGTDDKNSVNHVIHIDQPRLGLPSRDYYECTGIYKEACTAYVDFMISVARLIRQEERLPID
ENQLALEMNKVMELEKEIANATAKPEDRNDPMLLYNKMRLAQIQNNFSLEINGKPFSWLNFTNEI
MSTVNISITNEEDVVVYAPEYLTKLKPILTKYSARDLQNLMSWRFIMDLVSSLSRTYKESRNAFRK
ALYGTTSETATWRRCANYVNGNMENAVGRLYVEAAFAGESKHVVEDLIAQIREVFIQTLDDLTW
MDAETKKRAEEKALAIKERIGYPDDIVSNDNKLNNEYLELNYKEDEYFENIIQNLKFSQSKQLKKL
REKVDKDEWISGAAVVNAFYSSGRNQIVFPAGILQPPFFSAQQSNSLNYGGIGMVIGHEITHGFD
DNGRNFNKDGDLVDWWTQQSASNFKEQSQCMVYQYGNFSWDLAGGQHLNGINTLGENIADN
GGLGQAYRAYQNYIKKNGEEKLLPGLDLNHKQLFFLNFAQVWCGTYRPEYAVNSIKTDVHSPGN
FRIIGTLQNSAEFSEAFHCRKNSYMNPEKKCRVW

Figure 34 B

gattttaggt gatgggcaag tcagaaagtc agatggatat aactgatatc aacactccaa agccaaagaa gaaacagcga tggactcgac tggagatcag cctctcggtc cttgtcctgc tcctcaccat catagctgtg agaatgatcg cactctatgc aacctacgat gatggtattt gcaagtcatc agactgcata aaatcagctg ctcgactgat ccaaaacatg gatgccacca ctgagccttg tagagacttt ttcaaatatg cttgcggagg ctggttgaaa cgtaatgtca ttcccgagac cagctcccgt tacggcaact ttgacatttt aagagatgaa ctagaagtcg ttttgaaaga tgtccttcaa gaacccaaaa ctgaagatat agtagcagtg cagaaagcaa aagcattgta caggtcttgt ataaatgaat ctgctattga tagcagaggt ggagaacctc tactcaaact gttaccagac atatatgggt ggccagtagc aacagaaaac tgggagcaaa aatatggtgc ttcttggaca gctgaaaaag ctattgcaca actgaattct aaatatggga aaaaagtcct tattaatttg tttgttggca ctgatgataa gaattctgtg aatcatgtaa ttcatattga ccaacctcga cttggcctcc cttctagaga ttactatgaa tgcactggaa tctataaaga ggcttgtaca gcatatgtgg attttatgat ttctgtggcc agattgattc gtcaggaaga aagattgccc atcgatgaaa accagcttgc tttggaaatg aataaagtta tggaattgga aaaagaaatt gccaatgcta cggctaaacc tgaagatcga aatgatccaa tgcttctgta taacaagatg agattggccc agatccaaaa taacttttca ctagagatca atgggaagcc attcagctgg ttgaatttca caaatgaaat catgtcaact gtgaatatta gtattacaaa tgaggaagat gtggttgttt atgctccaga atatttaacc aaacttaagc ccattcttac caaatattct gccagagatc ttcaaaattt aatgtcctgg agattcataa tggatcttgt aagcagcctc agccgaacct acaaggagtc cagaaatgct ttccgcaagg ccctttatgg tacaacctca gaaacagcaa cttggagacg ttgtgcaaac tatgtcaatg ggaatatgga aaatgctgtg gggaggcttt atgtggaagc agcatttgct ggagagagta aacatgtggt cgaggatttg attgcacaga tccgagaagt ttttattcag actttagatg acctcacttg gatggatgcc gagacaaaaa agagagctga agaaaaggcc ttagcaatta aagaaaggat cggctatcct gatgacattg tttcaaatga taacaaactg aataatgagt acctcgagtt gaactacaaa gaagatgaat acttcgagaa cataattcaa aatttgaaat tcagccaaag taaacaactg aagaagctcc gagaaaaggt ggacaaagat gagtggataa gtggagcagc tgtagtcaat gcattttact cttcaggaag aaatcagata gtcttcccag ccggcattct gcagcccccc ttctttagtg cccagcagtc caactcattg aactatgggg gcatcggcat ggtcatagga cacgaaatca cccatggctt cgatgacaat ggcagaaact ttaacaaaga tggagacctc gttgactggt ggactcaaca gtctgcaagt aactttaagg agcaatccca gtgcatggtg tatcagtatg gaaacttttc ctgggacctg gcaggtggac agcaccttaa tggaattaat acactgggag aaaacattgc tgataatgga ggtcttggtc aagcatacag agcctatcag aattatatta aaaagaatgg cgaagaaaaa ttacttcctg gacttgacct aaatcacaaa caactatttt tcttgaactt tgcacaggtg tggtgtggaa cctataggcc agagtatgcg gttaactcca ttaaaacaga tgtgcacagt ccaggcaatt tcaggattat tgggactttg cagaactctg cagagtttc agaagccttt cactgccgca agaattcata catgaatcca gaaaagaagt gccgggtttg gtgatcttca aaagaagcat tgcagccctt ggctagactt gccaacacca cagaaatggg gaattctcta atcgaaagaa aatgggccct aggggtcact gtactgactt gagggtgatt aacagagagg gcaccatcac aatacagata acattaggtt gtcctagaaa gggtgtggag ggaggaaggg ggtctaaggt ctatcaagtc aatcatttct cactgtgtac ataatgctta atttctaaag ataatattac tgtttatttc tgtttctcat atggtctacc agtttgctga tgtccctaga aaacaatgca aaacctttga ggtagaccag gatttctaat caaaagggaa aagaagatgt tgaagaatag agttaggcac cagaagaaga gtaggtgaca ctatagttta aaacacattg cctaactact agtttttact tttatttgca acatttacag tccttcaaaa tccttccaaa gaattcttat acacattggg gccttggagc ttacatagtt ttaaactcat ttttgccata catcagttat tcattctgtg atcatttatt ttaagcactc ttaaagcaaa aaatgaatgt ctaaaattgt tttttgttgt acctgctttg actgatgctg agattcttca ggcttcctgc aattttctaa gcaatttctt gctctatctc tcaaaacttg gtatttttca gagatttata taaatgtaaa aataataatt tttatattta attattaact acatttatga gtaactatta ttataggtaa tcaatgaata ttgaagtttc agcttaaaat aaacagttgt gaaccaagat ctataaagcg atatacagat gaaaatttga gactatttaa acttataaat catattgatg aaaagattta agcacaaact ttagggtaaa aattgcgatt ggacagttgt ctagagatat atatacttgt ggttttcaaa ttggactttc aaaattaaat ctgtccctga gagtgtctct gataaaaggg caaatctgca cctatgtagc tctgcatctc ctgtcttttc aggtttgtca tcagatggaa atattttgat aataaattga aattgtgaac tcattgctcc ctaagactgt gacaactgtc taactttaga agtgcatttc tgaatagaaa tgggaggcct ctgatggacc ttctagaatt ataagtcaca aagagttctg gaaaagaact gttactgct tgataggaat tcatcttttg aggcttctgt tcctctcttt tcctgttgta ttgactattt tcgttcatta

Figure 34B (Continued)

cttgattaag attttacaaa agaggagcac ttccaaaatt cttatttttc ctaacaaaag atgaaagcag ggaatttcta tctaaatgat gagtattagt tccctgtctc ttgaaaaatg cccatttgcc tttaaaaaaa aaagttacag aaatactata acatatgtac ataaattgca taaagcataa gtatacagtt caataaactt aactttaact gaacaatggc cctgtagcca gcacctgtaa gaaacagagc agtaccagcg ctctaaaagc acctccttgt cactttatta ctcccagaac aacaactatc ctgacttcta atatcattca ctagctttgc ctggttttgt cttttatgca gatagaatca atcagtatgt attcttttgt gcctggcttc tttctctcag ccttacattt gtgagattcc tctgtattgt gctgattgtg gatcttttca ttctcattgc agaataatgt tctattgtgg gacttattac aatttgttca tcctattgtt gatgggcact tgagaacttt ccattttggc gctattacaa atagtgcaac tatgaatgta ctgcatgtta ccatcttact tgagccttta atggacttat ttcttcaaat ccttccaaaa attattataa gcattgaaat tatagtttca agccaactgt ggataccctt acccttcct cctttatcac aaccaccgtt acaagtatac ttatatttcc ctaaaataca tttaaaactt acctaagtga catttgtagt tggagtaata ggagcttcca gctctaataa aacagctgtc tctaacttat tttatttcca tcatgtcaga gcaggtgaag agccagaagt gaagagtgac tagtacaaat tataaaaagc cactagactc ttcactgtta gctttttaaa acattaggct cccatcccta tggaggaaca actctccagt gcctggatcc cctctgtcta caaatataag attttctggg cctaaaggat agatcaaagt caaaaatagc aatgcctccc tatccctcac acatccagac atcatgaatt ttacatggta ctcttgttga gttctataga gccttctgat gtctctaaag cactaccgat tctttggagt tgtcacatca gataagacat atctctaatt ccatccataa atccagttct actatggctg agttctggtc aaagaaagaa agtttagaag ctgagacaca aagggttggg agctgatgaa actcacaaat gatggtagga agaagctctc gacaataccc gttggcaagg agtctgcctc catgctgcag tgttcgagtg gattgtaggt gcaagatgga aaggattgta ggtgcaagct gtccagagaa aagagtcctt gttccagccc tattctgcca ctcctgacag ggtgaccttg ggtatttgca atattccttt gggcctctgc ttctctcacc taaaaaaaga gaattagatt atattggtgg ttctcagcaa gagaaggagt atgtgtccaa tgctgccttc ccatgaatct gtctcccagt tatgaatcag tgggcaggat aaactgaaaa ctcccattta agtgtctgaa tcgagtgaga caaaatttta gtccaaataa caagtaccaa agttttatca agtttgggtc tgtgctgctg ttactgttaa ccatttaagt ggggcaaaac cttgctaatt ttctcaaaag catttatcat tcttgttgcc acagctggag ctctcaaact aaaagacatt tgttattttg gaaagaagaa agactctatt ctcaaagttt cctaatcaga aatttttatc agtttccagt ctcaaaaata caaaataaaa acaaacgttt ttaatact

Figure 35A

MAHKQIYYSDKYFDEHYEYRHVMLPRELSKQVPKTHLMSEEEWRRLGVQQSLGWVHYMIHEPE
PHILLFRRPLPKDQQK

Figure 35B

agtctccggc gagttgttgc ctgggctgga cgtggttttg tctgctgcgc ccgctcttcg cgctctcgtt tcattttctg cagcgcgcca cgaggatggc ccacaagcag atctactact cggacaagta cttcgacgaa cactacgagt accggcatgt tatgttaccc agagaacttt ccaaacaagt acctaaaact catctgatgt ctgaagagga gtggaggaga cttggtgtcc aacagagtct aggctgggtt cattacatga ttcatgagcc agaaccacat attcttctct ttagacgacc tcttccaaaa gatcaacaaa aatgaagttt atctggggat cgtcaaatct ttttcaaatt taatgtatat gtgtatataa ggtagtattc agtgaatact tgagaaatgt acaaatcttt catccatacc tgtgcatgag ctgtattctt cacagcaaca gagctcagtt aaatgcaact gcaagtaggt tactgtaaga tgtttaagat aaaagttctt ccagtcagtt tttctcttaa gtgcctgttt gagtttactg aaacagttta cttttgttca ataaagtttg tatgttgcat ttaaaaaaaa aaaaaaa

Figure 36

gagctctcca tgcacacctg ttactgtttc tgtttttacc tgtaaatatc tgtctctgac ttccatgtct catgcacctc tatagggcaa agactgtgtc
ttaaacatca cggtagcctc agcatgttgt gcaatcaagg ttttttgtt tttgttcttt gttttttttt tggtattagc tttatttgta tcattttgaa atttttatca
aaaaagcagc gtgcctgctg tggttcccat cctctgggat ttaggaatct ttacccgatt ctccatccaa gtctgtcttt cgtattctag gctcttccta
aagttgtcat tcacatatac cctccagaat tttatagggt gtataatctg taacaactcg gaggaagcca attgcccttt agaaatatgg
ctgcaattgc ctcacttcct gtgtcatgtg actctcctag tcatcacatg acccatccac attgggaagc cagaattact tgcaggagta
acctagtgcc tatagctatg gcaggtacct gcatccttgt ttttgtttag tggatcctct atccttcaga gactctggaa cccctgtgct cttctcctca
tctagtgacc ctgaggtgat ggagttttca agtccttcca gagaggtaag agagagagct cccaatcagc attgtcacag tgcttctgga
atcctggcac tggaatttaa tgaatgacag actctctttg aatccagggc catcatggct ctttgagcaa ggcacagatg gaggggaggg
tcgaagttga aatgggtggg aagagtggtg gggagcatcc tgatttgggg tgggcagaga gttgtcatca gaagggttgc agggagagct
gcacccaggt gtctgtgggc cttgtcctaa tgaatgtggg agaccaggcc atgggcaccc aaaggcagct aagccctgcc cgggagagta
gttgaggggt ggagagggac ttgcttttca gtcattcctc attctgtcct caggaatgtc ccaagccttc gggtagggta agcatcatgg
ctggcagcct cacaggattg cttctacttc aggcagtgtc gtgggcatca gatgagtgag tcaaggcagt ggggaggtag cacagagcct
cccttctgcc tcatagtcct ttggtagcct tccagtaagc tggtggtaga cttttagtag gtgctcaata aatccttttg agtgactgag accaactttg
gggtgaggat ttttgaaacc gtcttcagtc tctccaaaca gctgtgtccg ttctccacat ccttgtcaga cctcacctct gcttgtgctc cctcctccc
aggtggtgcc cctgcatccc taaaagcttc agtacagctc ggtggtctgt gtctgcaatg ccacatactg tgactcttga ccccccgacc
tttcctgccc taggtgcctt cagccgctac aagagcagaa gcagtgggca ttggatggag ctgagtacag gaccatacag gctaattgca
ccggcacagg taaccattac accttcacc ccccgggcca ggctgggtcc tcctagaggt aaacggtgtc agtgatcacc atggagtttc
tccctgggca ctgataaccc tgtggatgtc ctcaggcctg ctactgatcc tgcagccaga agttccagaa agtgaaggga tttggagggg
ccgtgacaga tgcaggtgcc ctcaacatcc ttgccctgtc acccccctgcc cagaatttgc tacttaaatg gtacttctct gaagaagatg
aggaggaagg ggacaggatg acatagagcc actgacactt ttctttgcca attctttgga ccctgacttc tgcccatccc tgacatttgg
ttcctgtctt aatgccagtg aaataagatt tcgccgccta tcatctgcta actgctacgg actcaggctc agaaaggcct gcgcttcacc
caggtgccag cctccacagg ttccaaccca ggagcccaag ttccttttgg ccctgactca gacactatta ggactggcaa gtgataagca
gagtcccata ctctcctatt gactcggact accatatctt gatcatcctt ttctgtagga atcggatata acatcatctg ggtacccatg gccagctgtg
acttctccat ccgcacctac acctatgcag acacccctga tgatttccag ttgcacaact tcagcctccc agaggaagat accaagctca
aggtaggcat tctagctttt tcaggccctg agggccctga tgtctggggg ttgagaaact gtagggtagg tctgcttgta cagacatttt
gtcccctgct gttttgtcct gggggtggga gggtgggggc taatggctga accggatgca ctggttgggc tagtatgtgt tccaactctg
ggtgcttctc tcttcactac ctttgtctct agataccccc gattcaccga gccctgcagt tggcccagcg tcccgtttca ctccttgcca gcccctggac
atcacccact tggctcaaga ccaggggagc ggggaatggg aaggggccac tcaagggaca gcccagagac atctaccacc
agacctgggc cagatacatt gtgaagtaag ggatcaacaa ggatgtggga tcaggactgg cctccccttt ggccatgctg atctgtgtcc
caaccctcaa cctggttcca cttccagatc tgcctgtcct cagctcacct ttctaccttc tgggcctttc aaccttgggc ctgtcagtct tgcccactcc
atcaggcttc ctgttctctc ggtctggccc actttcttgg ctggatcatt catgaccttt ctcttgccag gttcctggat gcctatgctg agcacaagtt
acagttctgg gcagtgacag gtgaaaatga gccttctgct gggctgttga gtggataccc cttccagtgc ctgggcttca cccctgaaca
tcagcgagac ttcattgccc gtgacctagg tcctaccctt gccaacggta ctcaccacaa tgtccgccta ctcatgctgg atgaccaacg
cttgctgctg ccccactggg caaaggtggt aaggcctgga cctccatggt gctccagtga ccttcaaatc cagcatccaa atgattggct
cccaaactta gagggatttt tctacccaac tatggatccc tagagcacca ttccccggga cctccagggt gccatggatc ccacagttgg
gacttgaaac ctctctaggg ctgggggtgg tagctcatgg ctataattcc agcactttgg gaaccaaggt gggtggatca cttgaaccta
aggagttcaa gatgagcctg ggaaacatgg tgaaacccta actctacaaa aaaaaaaata gaaaagttag ccgggtgtgg tggtggcacg
ctatagtccc agtattctgg aggctaaggc gggaggttta gttgagccta ggaatttcag gctgcagtga gctatgattg tgccactgta
ctccagcctg tgtgacagag ggagaccctg tctcaaaaac aaaaacaaaa aatccctccc aaaacctctg tagttgcatt cttcccacca

Figure 36 (Continued)

cctaattcag gattcctaca agaggaacta gaagttccag aagcctgtgg gcagggtcca gggtgacttg ttcttcctt gcaggtactg
acagacccag aagcagctaa gtatgttcat ggtattgctg tacattggta cctggacttt ctggctccag ccaaagccac cctaagggag
acacaccacc tgttccccaa caccatgctc tttgcctcag aggcctgtgt gggttccaag ttctgggagc agagtgtgcg gctaggctcc
tgggatcgag ggatgcagta cagccacagc atcatcacag taagccaccc cagtctccct tcctgcaaag gagacctcag acccattagt
agtctcacca aagactgata gaagcccttc ctgtccagct ttccccaggt agcctgccct tttgcgcaac tctggggaac catgattccc
tgtcttgcct ttccttcaca ggtctgcaca cctcattgcc ccttttgcaa ctactgaggc acttgcagct gcctcagact tctcagctcc ccttgagatg
cctggatctt cacaccccca actccttagc tactaaggaa tgtgccctca cagggctgac ctacccacag ctgcctctcc cacatgtgac
ccttacctac actctctggg gacccccagt gttgagcctt tgtctctttg cctttgtcct taccctagaa cctcctgtac catgtggtcg gctggaccga
ctggaaccca tcattgtaga catcaccaag cacacgtttt acaaacagcc catgttctac caccttggcc acttcaggtg agtggagggc
gggcaccccc attccatacc aggcctatca tctcctacat cggatggctt acatcactct acaccacgag ggagcaggaa ggtgttcagg
gtggaacctc ggaagaggca cacccatccc cttttgcacc atggaggcag gaagtgacta ggtagcaaca gaaaacccca atgcctgagg
ctggactgcg atgcagaaaa gcagggtcag tgcccagcag catggctcca ggcctagaga gccagggcag agcctttgca ggagttatgg
ggtgggtccg tgggtgggcg acttcttaga tgagggtttc atgggaggta ccccgaggga ctctgaccat ctgttcccac attcagcaag
ttcattcctg agggctccca gagagtgggg ctggttgcca gtcagaagaa cgacccggac gcagtggcac tgatgcatcc cgatggctct
cctgttgtgg tcgtcctaaa ccggtgaggg caatggtgag gtctgggaag tgggctgaag acagcgttgg gggccttggc aggatcacac
tctcagcttc tcctccctgc tccctagctc ctctaaggat gtgcctctta ccatcaagga tcctgctgtg ggcttcctgg agacaatctc
acctggctac tccattcaca cctacctgtg gcgtcgccag tgatggagca gatactcaag gaggcactgg gctcagcctg ggcattaaag
ggacagagtc agctcacacg ctgtctgtga ctaaagaggg cacaacaggg ccagtgtgag cttacagcga cgtaagccca ggggcaatgg
tttgggtgac tcactttccc ctctaggtgg tgcccagggc tggaggcccc tagaaaaaga tcagtaagcc ccagtgtccc cccagccccc
atgcttatgt gaacatgcgc tgtgtgctgc ttgctttgga aactggcctg ggtccaggcc tagggtgagc tcactgtccg tacaaacaca
agatcagggc tgagggtaag gaaaagaaga gactaggaaa gctgggccca aaactggaga ctgtttgtct ttcctggaga tnnnnnnctg
ggcccgtgga gcagcagtgt cagcatcagg gcggaagcct taaagcagca gcgggtgtgc ccaggcaccc agatgattcc tatggcacca
gccaggaaaa atggcagctc ttaaaggaga aaatgtttga gcccagtcag tgtgagtggc tttattctgg gtggcagcac ccgtgtccgg
ctgtaccaac aacgaggagc acgggggcct ctggaagtca tgagagtaga aaaaccagtc ttggggagt

Figure 37A

MTEGTCLRRRGGPYKTEPATDLGRWRLNCERGRQTWTYLQDERAGREQTGLEAYALGLDTKN
YFKDLPKAHTAFEGALNGMTFYVGLQAEDGHWTGDYGGPLFLLPGLLITCHVARIPLPAGYREEI
VRYLRSVQLPDGGWGLHIEDKSTVFGTALNYVSLRILGVGPDDPDLVRARNILHKKGGAVAIPSW
GKFWLAVLNVYSWEGLNTLFPEMWLFPDWAPAHPSTLWCHCRQVYLPMSYCYAVRLSAAEDP
LVQSLRQELYVEDFASIDWLAQRNNVAPDELYTPHSWLLRVVYALLNLYEHHHSAHLRQRAVQK
LYEHIVADDRFTKSISIGPISKTINMLVRWYVDGPASTAFQEHVSRIPDYLWMGLDGMKMQGTNG
SQIWDTAFAIQALLEAGGHHRPEFSSCLQKAHEFLRLSQVPDNPPDYQKYYRQMRKGGFSFSTL
DCGWIVSDCTAEALKAVLLLQEKCPHVTEHIPRERLCDAVAVLLNMRNPDGGFATYETKRGGHL
LELLNPSEVFGDIMIDYTYVECTSAVMQALKYFHKRFPEHRAAEIRETLTQGLEFCRRQQRADGS
WEGSWGVCFTYGTWFGLEAFACMGQTYRDGTACAEVSRACDFLLSRQMADGGWGEDFESCE
ERRYLQSAQSQIHNTCWAMMGLMAVRHPDIEAQERGVRCLLEKQLPNGDWPQENIAGVFNKS
CAISYTSYRNIFPIWALGRFSQLYPERALAGHP

Figure 37B

ccettgccta ctgctcatgg gtgtggagac tgatattctg gaagactgat aggcagattt actattaaca aacacatagt ctgtggccca
gcaaagccac cccaatccct gcacaagggt aaaaggccag cattagagca ctgcagcagc aatgacggag ggcacgtgtc tgcggcgccg
agggggcccc tacaagaccg agcccgccac cgacctcggc cgctggcgac tcaactgcga gaggggccgg cagacgtgga
cctacctgca ggacgagcgc gccggccgcg agcagaccgg cctggaagcc tacgccctgg ggctggacac caagaattac tttaaggact
tgcccaaagc-ccacaccgcc tttgaggggg ctctgaacgg gatgacattt tacgtggggc tgcaggctga ggatgggcac tggacgggtg
attatggtgg cccacttttc ctcctgccag gcctcctgat cacttgccac gtggcacgca tccctctgcc agccggatac agagaagaga
ttgtgcggta cctgcggtca gtgcagctcc ctgacggtgg ctggggcctg cacattgagg ataagtccac cgtgtttggg actgcgctca
actatgtgtc tctcagaatt ctgggtgttg ggcctgacga tcctgacctg gtacgagccc ggaacattct tcacaagaaa ggtggtgctg
tggccatccc ctcctggggg aagttctggc tggctgtcct gaatgtttac agctgggaag gcctcaatac cctgttccca gagatgtggc
tgtttcctga ctgggcaccg gcacacccct ccacactctg gtgccactgc cggcaggtgt acctgcccat gagctactgc tacgccgttc
ggctgagtgc cgcggaagac ccgctggtcc agagcctccg ccaggagctc tatgtggagg acttcgccag cattgactgg ctggcgcaga
ggaacaacgt ggcccccgac gagctgtaca cgccccacag ctggctgctc cgcgtggtat atgcgctcct caacctgtat gagcaccacc
acagtgccca cctgcggcag cgggccgtgc agaagctgta tgaacacatt gtggccgacg accgattcac caagagcatc agcatcggcc
cgatctcgaa aaccatcaac atgcttgtgc gctggtatgt ggacgggccc gcctccactg ccttccagga gcatgtctcc agaatcccgg
actatctctg gatgggcctt gacggcatga aaatgcaggg caccaacggc tcacagatct gggacaccgc attcgccatc caggctctgc
ttgaggcggg cgggcaccac aggcccgagt tttcgtcctg cctgcagaag gctcatgagt tcctgaggct ctcacaggtc ccagataacc
ctcccgacta ccagaagtac taccgccaga tgcgcaaggg tggcttctcc ttcagtacgc tggactgcgg ctggatcgtt tctgactgca
cggctgaggc cttgaaggct gtgctgctcc tgcaggagaa gtgtccccat gtcaccgagc acatccccag agaacggctc tgcgatgctg
tggctgtgct gctgaacatg agaaatccag atggagggtt cgccacctat gagaccaagc gtgggggtca cttgctggag ctgctgaacc
cctcggaggt cttcggggac atcatgattg actacaccta tgtggagtgc acctcagccg tgatgcaggc gcttaagtat ttccacaagc
gtttcccgga gcacagggca gcggagatcc gggagaccct cacgcagggc ttagagttct gtcggcggca gcagagggcc gatggctcct
gggaaggctc ctggggagtt tgcttcacct acggcacctg gtttggcctg gaggccttcg cctgtatggg gcagacctac cgagatggga
ctgcctgtgc agaggtctcc cgggcctgtg acttcctgct gtcccggcag atggcagacg gaggctgggg ggaggacttt gagtcctgcg
aggagcggcg ttatttgcag agtgcccagt cccagatcca taacacatgc tgggccatga tggggctgat ggccgttcgg catcctgaca
tcgaggccca ggagagagga gtccggtgtc tacttgagaa acagctcccc aatggcgact ggccgcagga aaacattgct ggggtcttca
acaagtcctg tgccatctcc tacacgagct acaggaacat cttccccatc tgggccctcg gccgcttctc ccagctgtac cctgagagag
cccttgctgg ccacccctga gaacatgcct acctgctggg tgccgtctgt gcgttccagt gaggccaagg ggtcctggcc gggttgggga
gccctcccat aaccctgtct tgggctccaa cccctcaacc tctatctcat agatgtgaat ctgggggcca ggctggaggc agggatgggg
acagggtggg tggcttagac tcttgatttt tactgtaggt tcatttctga aagtagcttg tcgggcttgg gtgaggaagg gggcacagga
gccgtgaccc ctgaggaggc acagcgcctt ctgccacctc tgggcacggc ctcaaggtag tgaggctagg aggttttttc tgaccaatag
ctgagttctt gggagaggag cagctgtgcc tgtgtgattc cttagtgtcg agtgggctct gggctggggt cggccctggg caggcttctc
ctgcaccttt tgtctgctgg gctgagggac acgagggcaa ccctgtgaca atggcaggta gtgtgcatcc gtgaatagcc cagtgcgggg
gttgctcatg gagcatcctg aggccgtgca gcagggagcc ccatgccccc gggtcgtgag cttgcctgcg tatggggtgg tgtcatggag
cctcatgccc ctgggtcgtg agctcgcctg agtatggggt ggtgtcatgg agccgcatac ccctgggttg tgagctcgcc tgcatatgca
gggtctgtca tggaacatcc caagtctgtg cagcagggag ccccatgccc ctgggacatg aacccacctg cgtggaatgc tgtttgtgag
gtgtctacag ggtttatagt agtcttgtgg acacagaaat gcacagggga cacttacgga cacaga

Figure 38

gacgacgact tgctgttcga ggatgtgtac gagctgtgcg aggtgatcgg aaagggtccc ttcagtgttg tacgacgatg tatcaacaga gaaactgggc aacaatttgc tgtaaaaatt gttgatgtag ccaagttcac atcaagtcca gggttaagta cagaagatct aaagcgggaa gccagtatct gtcatatgct gaaacatcca cacattgtag agttattgga gacatatagc tcagatggaa tgctttacat ggttttcgaa ttgtgagtgt gtattttaat tcttaaaggg taaaacttga agcaatgttg gtgttggata atgctaacac ttttctcttg aaatttagca gtagttgtga acttatctgt tcagaaagac ctaaagtcac aagaaaaaag gattatgtca tcataaggtt tacagtggca aaggaagcaa aagctgggca tattcagtta ctcttcatgc tttcagcatg cttcagagaa gagact

Figure 39

gagcctcaaa tatctccaaa atctgatacc aatccttttg attgtgaatt atattctgta gctaccaaag aaggaagaag aaaactagga aggagtaagc acaaagatct cttcacattc tccgggactg cggtaccaaa tatcagcaca gcacttcttg aaaaaggatg tagattttaa tctgaacttt gaaccatcac tgaggtggcc cgccggtttc tgagccttc

Figure 40A

MDGKVAVQERGPPAVSWVPEEGEKLDQEDEDQVKDRGQWTNKMEFVLSVAGEIIGLGNVWRF
PYLCYKNGGGAFFIPYFIFFFVCGIPVFFLEVALGQYTSQGSVTAWRKICPLFQGIGLASVVIESYL
NVYYIIILAWALFYLFSSFTSELPWTTCNNFWNTEHCTDFLNHSGAGTVTPFENFTSPVMEFWER
RVLGITSGIHDLGSLRWELALCLLLAWVICYFCIWKGVKSTGKVVYFTATFPYLMLVILLIRGVTLP
GAYQGIIYYLKPDLFRLKDPQVWMDAGTQIFFSFAICQGCLTALGSYNKYHNNCYKDCIALCFLNS
ATSFVAGFVVFSILGFMSQEQGVPISEVAESGPGLAFIAFPKAVTMMPLSQLWSCLFFIMLIFLGL
DSQFVCVECLVTASIDMFPRQLRKSGRRELLILTIAVMCYLIGLFLVTEGGMYIFQLFDYYASSGIC
LLFLSLFEVVCISWVYGADRFYDNIEDMIGYRPWPLVKISWLFLTPGLCLATFLFSLSKYTPLKYNN
VYVYPPWGYSIGWFLALSSMVCVPLFVVITLLKTRGPFRKRLRHVITPDSSLPQPKQHPCLDGSA
GRNFGPSPTREGLIAGEKETHL

Figure 40B

gtaccggttc ggaattcccg ggtcgaccca cgcgtccgga aggctacaga gagagccagg ttttggtgcc atgcacacag ggaaacttag
agttcagaga gggggtgtga tttgcctgac ctcacacagc aagttagaga cccagctcca cgactcattg tcttgctgcc cagagctgct
ggctcccctg tttactctga gctgatcgat caccttagca cacagctggc taggagagaa ccatgcagtc acttcggcca cacctgcccg
ttgacccttg ctacctcggc aggctttgat cccttctgac ctggaggcca gaggctaggc tgaggtcact cagcagacat caaggacctg
ggcagatggg ccggctggga tggtggcgag ctgtacagat aaaaagggac atgaaaatga aaagcccgag cctgagtttt catcacggtt
ccactcctga gtggtcttgg gtgaatcact tcatctgcca aggcctggat ttcctcatct gcaaactcag aaaactaagg ctttggccct
cgtcatcctg cccacccagc ggggcttccc aacccaccac acagccatgg acgggaaggt ggcagtgcaa gagcgtgggc ctcctgcggt
ctcctgggtc cccgaggagg gagagaagtt ggaccaggaa gacgaggacc aggtgaagga tcggggccaa tggaccaaca
agatggagtt tgtgctgtca gtggccgggg agatcattgg gctgggcaat gtctggaggt ttccctatct ctgctacaaa aacggaggtg
gagccttctt catcccctac ttcatcttct tctttgtctg cggcatcccg gtgttcttcc tggaggtggc gttgggccaa tacaccagcc aagggagtgt
cacagcctgg aggaagatct gccccctctt ccagggcatt ggtctggcat ctgtggtcat cgagtcatat ttgaatgtct actacatcat
catccttgcc tgggctctct tctacctgtt cagctccttc acttctgagc tgccctggac gacctgcaac aacttttgga acacagagca
ttgcacggac tttctgaacc actcaggagc cggcacagtg accccatttg agaattttac ctcacctgtc atggaattct gggagagacg
agttctgggc atcacctcgg gcatccatga cctgggctcc ctgcgctggg agctggccct gtgcctcctg ctcgcctggg tcatctgcta
tttctgcatc tggaaggggg tcaagtccac aggcaaggtg gtttatttca cagccacgtt tccgtacctg atgcttgtca ttttgctgat cagaggtgtc
acccttcccg gagcctacca gggcatcatc tactacttga agccagattt gttccgcctc aaggaccctc aggtgtggat ggatgcgggc
acccagatct tcttctcctt tgccatctgc caggggtgcc tgacagccct gggcagctac aacaagtatc acaacaactg ctacaaggac
tgcatcgccc tctgcttcct gaacagtgcc accagctttg tggctgggtt tgttgtcttc tccatcctgg gcttcatgtc ccaagagcaa
ggggtgccca tttctgaagt ggccgagtca ggtcctgggc tggccttcat cgccttcccc aaggctgtga ctatgatgcc cttatcccag
ctgtggtcct gcctgttctt tatcatgctc atattcctag ggctggacag ccagtttgtc tgtgtggagt gcctggtgac agcctccata gacatgttcc
ccaggcagct ccggaagagc gggcggcgcg agctcctcat cctcaccatc gccgtcatgt gctacctgat agggcttttc ctggtcaccg
agggcgggat gtacatcttc cagctgtttg actactatgc ttccagtggc atatgcctgc tgttcctgtc attgtttgaa gtggtctgca taagctgggt
gtatggggcg gaccgtttct atgacaacat tgaggacatg attggctacc ggccatggcc cctggtgaag atctcctggc tcttcctgac
ccctggactt tgcctggcca ctttcctctt ctccttgagc aagtacaccc ccctcaagta caacaacgtc tatgtgtacc cgccctgggg
atactccatt ggctggttcc tggctctgtc ctccatggtc tgtgtcccac tcttcgtcgt catcaccctc ctgaagactc ggggtccttt caggaagcgt
ctgcgtcacg tcatcacccc tgactccagt ctgccacagc ccaagcaaca tccctgcttg gatggcagtg ctggccggaa ctttgggccc
tccccaacaa gggaaggact gatagccggg gagaaggaga cccatttgta gggtgtggcc agagcgaggc ggctcctaag ccgggaacct
aggtcagggc caccctccat tctcagcgga cagcctctgc ctctgtctcc tgccacaatc ctgctgggaa ctctggagag ccacaggcac
ccccagctgg aggccagact cctctcttgt gctagctgga gcagctcctt cccctttgct gataacacca ccactgggac gtgccatgtt
gggacgccac tccctgtgga aggcaccatc gtttttataa agggggggtct ttttggaggc cgccatctga ttgcaacacc tcgagttatg
aggattccac tgtggggatg cctcttgtta gagcgtactg catttgtaca cggggagagg agctataatt ggaacgcaca ctgccgtcca
atgtggagag cctgatggga caataccctg ttggaagtga caactgaaca cactgtgttg gatcggaggt tccgttaggg gatccttcct
taggcttaac gacagaggca agcctttgca tgccgtcagt ctggagtttc ctccgagtct ctcatggcat ctccagctcc tgccctagtt
ccgcactgtt cttgcagtgt ttcatcaact cctggagcat tggaatggaa ggggcttggg agatgattcc tagacttcac aaacactcgg
catgcctccc tgcactgtcc gttcctctgc ccaaggccga tattgctaac tgatcacaga ttctttccca cctcacaatc ctccgaatgt
gctccaggcg acaccatttg ccatcctgct tctaacgcaa acccctgact tcatggatga ggaacctgga gaccaaagag acaaagggac
tttttcaagt tcacatgggg acccccttct tgggggccag agatatgact aaaaccttat ctccttgtgc tcaggccagt gtcttcccat
taacccccctg ccttagttaa caagtgtgta tggattgcca

Figure 41A

MGTQKVTPALIFAITVATIGSFQFGYNTGVINAPEKIIKEFINKTLTDKGNAPPSEVLLTSLWSLSVAI
FSVGGMIGSFSVGLFVNRFGRRNSMLIVNLLAVTGGCFMGLCKVAKSVEMLILGRLVIGLFCGLC
TGFVPMYIGEISPTALRGAFGTLNQLGIVVGILVAQIFGLEFILGSEELWPLLLGFTILPAILQSAALP
FCPESPRFLLINRKEEENAKQILQRLWGTQDVSQDIQEMKDESARMSQEKQVTVLELFRVSSYR
QPIIISIVLQLSQQLSGINAVFYYSTGIFKDAGVQEPIYATIGAGVVNTIFTVVSLFLVERAGRRTLHM
IGLGGMAFCSTLMTVSLLLKDNYNGMSFVCIGAILVFVAFFEIGPGPIPWFIVAELFSQGPRPAAM
AVAGCSNWTSNFLVGLLFPSAAHYLGAYVFIIFTGFLITFLAFTFFKVPETRGRTFEDITRAFEGQA
HGADRSGKDGVMEMNSIEPAKETTTNV

Figure 41B

gtggggtggg gtggggctgg gggcttgtcg cctttcagg ctccaccctt tgcggagattataaatagtc atgatcccag cgagacccag agatgcctgt aatggtgaga ctttggatcc ttcctgagga cgtggagaaa actttctgct gagaaggaca ttttgaaggt tttgttggct gaaaaagctg tttctggaat caccctaga tctttcttga agacttgaat tagattacag cgatggggac acagaaggtc accccagctc tgatatttgc catcacagtt gctacaatcg gctctttcca atttggctac aacactgggg tcatcaatgc tcctgagaag atcataaagg aatttatcaa taaaactttg acggacaagg gaaatgcccc accctctgag gtgctgctca cgtctctctg gtccttgtct gtggccatat tttccgtcgg gggtatgatc ggctcctttt ccgtcggact cttcgtcaac cgctttggca ggcgcaattc aatgctgatt gtcaacctgt tggctgtcac tggtggctgc tttatgggac tgtgtaaagt agctaagtcg gttgaaatgc tgatcctggg tcgcttggtt attggcctct tctgcggact ctgcacaggt tttgtgccca tgtacattgg agagatctcg cctactgccc tgcggggtgc ctttggcact ctcaaccagc tgggcatcgt tgttggaatt ctggtggccc agatctttgg tctggaattc atccttgggt ctgaagagct atggccgctg ctactgggtt ttaccatcct tcctgctatc ctacaaagtg cagcccttcc attttgccct gaaagtccca gatttttgct cattaacaga aaagaagagg agaatgctaa gcagatcctc cagcggttgt ggggcaccca ggatgtatcc caagacatcc aggagatgaa agatgagagt gcaaggatgt cacaagaaaa gcaagtcacc gtgctagagc tctttagagt gtccagctac cgacagccca tcatcatttc cattgtgctc cagctctctc agcagctctc tgggatcaat gctgtgttct attactcaac aggaatcttc aaggatgcag gtgttcaaga gcccatctat gccaccatcg gcgcgggtgt ggttaatact atcttcactg tagtttctct atttctggtg gaaagggcag gaagaaggac tctgcatatg ataggccttg gagggatggc tttttgttcc acgctcatga ctgtttcttt gttattaaag gataactata atgggatgag ctttgtctgt attggggcta tcttggtctt tgtagccttc tttgaaattg gaccaggccc cattccctgg tttattgtgg ccgaactctt cagccagggc cccgcccag ctgcgatggc agtggccggc tgctccaact ggacctccaa cttcctagtc ggattgctct tcccctccgc tgctcactat ttaggagcct acgtttttat tatcttcacc ggcttcctca ttaccttctt ggcttttacc ttcttcaaag tccctgagac ccgtggcagg acttttgagg atatcacacg ggcctttgaa gggcaggcac acggtgcaga tagatctgga aaggacggcg tcatggagat gaacagcatc gagcctgcta aggagaccac caccaatgtc taagtcgtgc ctccttccac ctcccctccg gcatgggaaa gccacctctc cctcaacaag ggagagacct catcaggatg aacccaggac gcttctgaat gctgctactt aattcctttc tcatcccacg cactccatga gcaccccaag gctgcggttt gttggatctt caatggcttt ttaaatttta tttcctggac atcctcttct gcttaggaga gaccgagtga acctaccttc atttcaggag ggattggccg cttggcacat gacaactttg ccagcttttc ctcccttggg ttctgatatt gccgcactag gggatatagg agaggaaaag taaggtgcag ttcccccaac ctcagactta ccaggaagca gatacatatg agtgtggaag ccggagggtg tttatgtaag agcaccttcc tcacttccat acagctctac gtggcaaatt aacttgagtt ttatttattt tatcctctgg tttaattaca taatttttt ttttttactt taagtttcag gatacatgtg ccgaatgtgc aggtttgtta cataggtata tatatgccat gatggaaata tttatttttt taagcgtaat tttgccaaat aataaaaaca gaaggaaatt gagattagag ggaggtgttt aaagagaggt tatagagtag aagatttgat gctggagagg ttaaggtgca ataagaattt agggagaaat gttgttcatt attggagggt aaatgatgtg gtgcctgagg tctgtacgtt acctcttaac aatttctgtc cttcagatgg aaactcttta acttctcgta aaagtcatat acctatataa taaagctact gatttccttg gagcttttt ctttaagata atagtttaca tgtagtagta cttgaaatct aggattatta actaatatgg gcattgtagt taatgatggt tgatgggttc taattttgga tggagtccag ggaagagaaa gtgatttcta gaaagcctgt tcccctcact ggatgaaata actccttctt gtagtagtct cattactttt gaagtaatcc cgccacctat ctcgtgggag agccatccaa ataagaaacc taaaataatt ggttcttggt agagattcat tatttttcca ctttgttctt taggagattt taggtgttga ttttctgttg tattttaact cataccttta aaggaattcc ccaaagaatg tttatagcaa acttggaatt tgtaacctca gctctgggag aggatttttt tctgagcgat tattatctaa agtgtgttgt tgctttaggc tcacggcacg cttgcgtatg tctgttacca tgtcactgtg gtcctatgcc gaatgccctc aggggacttg aatctttcca ataaaccagg tttagacagt atgagtcaat gtgcagtgta gcccacactt gagaggatga atgtatgtgc actgtcactt tgctctgggt ggaagtacgt tattgttgac ttatttttctc tgtgtttgtt cctacagccc ctttttcata tgttgctcag tctccctttc ccttcttggt gcttacacat ctcagaccct ttagccaaac ccttgtcagt gacagtattt tggttcttag ttctcactgt tccctctgct cctggagcct ttgaataaaa atgcacgtag ctgaggccgg atgcggtggc tcacgcctgt aatcccagca ctttgggagg cctaggcggg cggtcagggg ttcgagacca gtctggccaa catcgtgaaa ccctgtctct actaaaaatg caaaaattag ccgggcgtgg tggcgggcgc ctgtaatccc agctacttgg

Figure 41B (Continued)

gaagctgagg cgggagaatc atgtgaaccc gggacgcagg ggttgcagtg agcggagatc gcatcattgc actctagcct gggccacagg gcgagactcc gtctcaaaaa aaaaaaaatg cacatagcta tcgagtgtgc tttagcttga aaaggtgacc ttgcaacttc atgtcaactt tctggctcct caaacagtag gttggcagta aggcagggtc ccatttctca ctgagaagat tgtgaatatt tccatatgga ttttctattg ttactctggt tctttgtttt aaaataaaaa ttctgaatgt acacg

Figure 42

gccgagagcg ggatccgcgc tccctcggtc cttcctccct cccctcttgc ggcctcccgc tgtcatctgg agccgctcct gccgcccccc
ggcggcgacc gcgggaaggg ccggccccca tccgcacccc tgaccccgga ggtcaacaac gggatggtcc ctgggtccca
ggggaagaga catcacccag taggagggag tacggtctag acagaggcca cgagggcggg agggggcgag agtggagagt
ggcccagctg gccagggtcg tctaagtgag aggaaaaggg agagggcggt tgagaccagg ccctgaattc cgcgttcatc ttatcctgag
gtctgtgggg acctgttgaa ggactggggc aggggacgga cgcgggcatc cttccatttg gaacagccat tccggcagca tcaggatggg
gcggaggcaa agcggggagt gggcgaggca agtggtgctg taaacctgtg cgagaagggg gcggtgactc taagggcagg
aaggagccct ggtcacacac acactcccac gcaaggtatt cagtgccgag tgtggccttg gtgctaggat tcaaagagga aaggaagaaa
actttccatt ctaaaagaaa ctccacgtga ggcgaagaag atgaaatata gtcagaaaac cataccagta ggtggtaggt aaatgcagaa
gtatttaaga gctcatacag gagtacctgc ctcaggacag ggaatctgag atgctctgca gagctggatc ttaaagaacg gattaagttg
ataaatgatg catacgacat cctatagaag actgtcacca ccccacctca ctgatcagcc ctctgcctac agccacaccc acagaacatt
cagccatttc tcctgtggtt cccagcctgc agcacagcgt ctgcacgtgg tgagggctcc caaatctctg aggaatgaat ggctcactgc
tgagagcaac cttctgggag ttcagggcat gtggaactgg aataagagca acgtgtgaac agattacagg aaggcaatgc aggacagctg
gacccttgg ggggaaactt atggggaaca cagttttctt acttataagc actgagattg cctgagaggg gagagatgat gcagagtgtc
ccaaatgtat ttgacgtgga atcctttac cagcgaaaca tctcttgggc ctagagttct gaaaattgca ctttgaaaaa cactgctctc
agctcttaca aacctcgtgt tttcatccta actgtgcttc agagaattgt gtcccagcag gaaaggaaac ccccgtataa ttccctacgt
tgctagcatt tggaagtggg atgaaaatgg acctacattc aaacccctaa tttcaagctc tgaaggaggt accaggatcc atcacgatgc
ttgtaaaatt gttcctttca ttttcctggt tgtaaactta tgcagactgt aaagtcaagg caagcctgtg cccagcaaat aataagaaaa
taagggaggc atggtaaaca aaacaaaaca ctttgagagt tggtcaactt gctgttattt tgagctgttt ctaaaaatct cagggtagat
tccctcccct gcttgcctcc ttaacccagt ccttttctt cctcctccag gaactctgga atgctgacct acagtctgag ttcctgtgcc cttgcctggg
gctgactctc tacttgacct gtaacccaca actgggcaag agaaaattct gcagccacag ctctgaggac atgagcaaaa tggtttccag
acggaatgtc aaggattccc atgaagtgtc agggagtctt caggccacac ttcaggttat ctccttctct ttccctttc tgcttcacac ttgctctcat
cctctttctc accccacatc tggtcagagg agataggaag gttcttgtta cctggtacaa gacaactttt ggaatcccag atattcagat
tatttcccct tttcctcttt gcaaatgata atactcacag ccaagaggca tcacccggat ccgaggctgc ttctccttag gacatgcaga
cagaaggaga aggcggggct ggcagcctcc agtccacagc ctccctttct tcttccggcg atgattaact aggcctagac aatggagatc
tacggcatac gcccagggcc tcctcttctc aagcatggct gatcagtcac tttcccgtct atccttcatt tattgaagcc aactatgaac
gactaaagga aggatgagaa aagtcaccca aagtcaaagg ggacagcgtg ggagactgtt ctagacagaa ggaaacacct
ttgcaaagac cctgaggaag gcaggggact ctccaggagc agaagggctg tgtggcttca gagtccacaa aagagagcag atacaggaca
ctggctagag caggccctgg agcccgctgc tgtcctggag gccttgggga ggcccagtgt tcccagggtg gaagaagtag gggacagctt
gacgtagtgg ctgttgatca gctgatatgg aagtatgtca ttttattaac aattgagaaa ggagtgctgt gcaattccat tcaatgccag
tgatgcttat ggccgttttt atgagttctg tcattttcaa atgagcaaga ggaagcctct aaggggttt aagcagggac tgacgtaatc
agatctgtgt ttttccaaag ggagagggag aaaaagaaca tttcttattt ttcaaaaaag gtaatgcaaa agcatcattc cacaattctc
ttgtaatgaa aaaaataaat gcaaacttaa gcaaatccat cattctgaaa gaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
aaaaaaaaaa aa

Figure 43A

MPLLWLRGFLLASCWIIVRSSPTPGSEGHSAAPDCPSCALAALPKDVPNSQPEMVEAVKKHILN
MLHLKKRPDVTQPVPKAALLNAIRKLHVGKVGENGYVEIEDDIGRRAEMNELMEQTSEIITFAESG
TARKTLHFEISKEGSDLSVVERAEVWLFLKVPKANRTRTKVTIRLFQQQKHPQGSLDTGEEAEEV
GLKGERSELLLSEKVVDARKSTWHVFPVSSSIQRLLDQGKSSLDVRIACEQCQESGASLVLLGKK
KKKEEEGEGKKKGGGEGGAGADEEKEQSHRPFLMLQARQSEDHPHRRRRRGLECDGKVNICC
KKQFFVSFKDIGWNDWIIAPSGYHANYCEGECPSHIAGTSGSSLSFHSTVINHYRMRGHSPFANL
KSCCVPTKLRPMSMLYYDDGQNIIKKDIQNMIVEECGCS

Figure 43B

tccacacaca caaaaaacct gcgcgtgagg ggggaggaaa agcagggcct ttaaaaaggc aatcacaaca acttttgctg ccaggatgcc
cttgctttgg ctgagaggat ttctgttggc aagttgctgg attatagtga ggagttcccc caccccagga tccgaggggc acagcgcggc
ccccgactgt ccgtcctgtg cgctggccgc cctcccaaag gatgtaccca actctcagcc agagatggtg gaggccgtca agaagcacat
tttaaacatg ctgcacttga agaagagacc cgatgtcacc cagccggtac ccaaggcggc gcttctgaac gcgatcagaa agcttcatgt
gggcaaagtc ggggagaacg ggtatgtgga gatagaggat gacattggaa ggagggcaga aatgaatgaa cttatggagc agacctcgga
gatcatcacg tttgccgagt caggaacagc caggaagacg ctgcacttcg agatttccaa ggaaggcagt gacctgtcag tggtggagcg
tgcagaagtc tggctcttcc taaaagtccc caaggccaac aggaccagga ccaaagtcac catccgcctc ttccagcagc agaagcaccc
gcagggcagc ttggacacag gggaagaggc cgaggaagtg ggcttaaagg gggagaggag tgaactgttg ctctctgaaa aagtagtaga
cgctcggaag agcacctggc atgtcttccc tgtctccagc agcatccagc ggttgctgga ccagggcaag agctccctgg acgttcggat
tgcctgtgag cagtgccagg agagtggcgc cagcttggtt ctcctgggca agaagaagaa gaaagaagag gagggggaag
ggaaaaagaa gggcggaggt gaaggtgggg caggagcaga tgaggaaaag gagcagtcgc acagaccttt cctcatgctg
caggcccggc agtctgaaga ccaccctcat cgccggcgtc ggcggggctt ggagtgtgat ggcaaggtca acatctgctg taagaaacag
ttctttgtca gtttcaagga catcggctgg aatgactgga tcattgctcc ctctggctat catgccaact actgcgaggg tgagtgcccg
agccatatag caggcacgtc cgggtcctca ctgtccttcc actcaacagt catcaaccac taccgcatgc ggggccatag ccccttgcc
aacctcaaat cgtgctgtgt gcccaccaag ctgagaccca tgtccatgtt gtactatgat gatggtcaaa acatcatcaa aaaggacatt
cagaacatga tcgtggagga gtgtgggtgc tcatagagtt gcccagccca gggggaaagg gagcaagagt tgtccagaga agacagtggc
aaaatgaaga aatttttaag gtttctgagt taaccagaaa aatagaaatt aaaaacaaaa caaaacaaaa aaaaaaacaa aaaaaaacaa
aagtaaatta aaaacaaacc tgatgaaaca gatgaaacag atgaaggaag atgtggaaat cttagcctgc cttagccagg gctcagagat
gaagcagtga agagacagat tgggagggaa agggagaatg gtgtaccctt tatttcttct gaaatcacac tgatgacatc agttgtttaa
acggggtatt gtcctttccc cccttgaggt tcccttgtga gcttgaatca accaatctga tctgcagtag tgtggactag aacaacccaa
atagcatcta gaaagccatg agtttgaaag ggcccatcac aggcactttc ctagcctaat

Figure 44

MGLAEYFGFD DHDTDLRTEL VAGLTTFLAM SYIVLVNPVV MTQRTTAGEV VKPGIALANY
SHDQTVQMLA VVTLLASGVA MLVMAFYANR PFALAPGLGL NAFFAFTVVG TLGVPWQTAL
AAVFTEGLLF IVLTAVGARE YVITLFPEPV KLAVGTGIGL YLAIIGLEAM GIVVGDAGTI
LALGNLAQNP VAVVSILGLF FTIALHARGV TGSIVLGIIA TAATGGVLTF AGVVDPGVLI
GDFVRTGGIA TQRLPHAQYD ITPLVGAFLA GFQDIDAFSF ALIVFTFFFV DFFDTAGTLV
GVGQAGGFLN TDGNLPDADE PLMADAIGTT FGAIIGTSTV TTYIESATGV EEGGRTGMVA
LVVAVLFFLS LLVVPLAAAI PQYASHIALV VVALLMLANV TAIDWDDITH SIPAGLTIIV
MPFTYSIAYG IAAGIVSYPV VKVATGDADE VAIGQWLLAA AFIVYFYVRT SGVLAAAV

专利名称(译)	用于诊断和治疗妊娠并发症的核酸和多肽		
公开(公告)号	EP1839057A4	公开(公告)日	2008-12-31
申请号	EP2005858653	申请日	2005-12-15
申请(专利权)人(译)	贝斯以色列女执事医疗中心		
当前申请(专利权)人(译)	贝斯以色列女执事医疗中心		
[标]发明人	KARUMANCHI S ANANTH SUKHATME VIKAS P		
发明人	KARUMANCHI, S., ANANTH SUKHATME, VIKAS, P.		
IPC分类号	G01N33/53 A61B5/055 C12Q1/68		
CPC分类号	G01N33/689 C12Q1/6883 C12Q2600/158 G01N33/6893 G01N2800/321 G01N2800/368		
优先权	60/636275 2004-12-15 US		
其他公开文献	EP1839057B1 EP1839057A2		
外部链接	Espacenet		

摘要(译)

本文公开了用于诊断或治疗妊娠相关的高血压病症的方法，其包括使用多肽或编码选自以下的多肽的核酸：卵泡抑素相关蛋白，白细胞介素8，抑制素A，VEGF-C，血管生成素，β受精，假设蛋白，白细胞相关Ig样受体分泌蛋白，红细胞分化蛋白，脂肪形成抑制因子，促肾上腺皮质激素释放因子结合蛋白，α-1抗胰凝乳蛋白酶，胰岛素样生长因子结合蛋白-5，CD33L，细胞因子受体样因子1 ，血小板衍生的内皮生长因子，赖氨酰羟化酶异构体2，斯钙素前体，分泌的卷曲相关蛋白，半乳糖凝集素-3，α防御素，ADAM-TS3，胆囊收缩素前体，干扰素刺激的T细胞α化学引诱物前体，azurocidin，精氨酸氧化酶，UDP糖基转移酶2家族多肽B28，神经营养酪氨酸激酶受体2，中性内肽idase，CDC28蛋白激酶调节亚基2，β葡萄糖苷酶，羊毛甾醇合成酶，钙/钙调蛋白依赖性丝氨酸蛋白激酶，雌激素受体 - 可变剪接转录物H，趋化因子（CX3C基序）受体1，酪氨酸酶相关蛋白1，羟基-δ- 5-甾体脱氢酶，二氢吡喃酶类似物-4和细胞色素P450-家族11。

Table 1: Clinical characteristics of the study patients

	Normal (n=15)	Pre-eclampsia (n=19)
Maternal Age (years)	35.2	31.9
Gestational Age (wks)	39.0	31.1*
Primiparous (%)	19	81*
Systolic BP (mm Hg)	107	167.2**
Diastolic BP (mm Hg)	83	101.8**
Proteinuria (g protein/g creat)	<0.3	5.2**
Serum Uric Acid (mg/dl)	NA	6.8
Hematocrit (%)	35.7	33.9
Platelet Count (K/µl)	217	198
Serum Creatinine (mg/dl)	0.5	0.6