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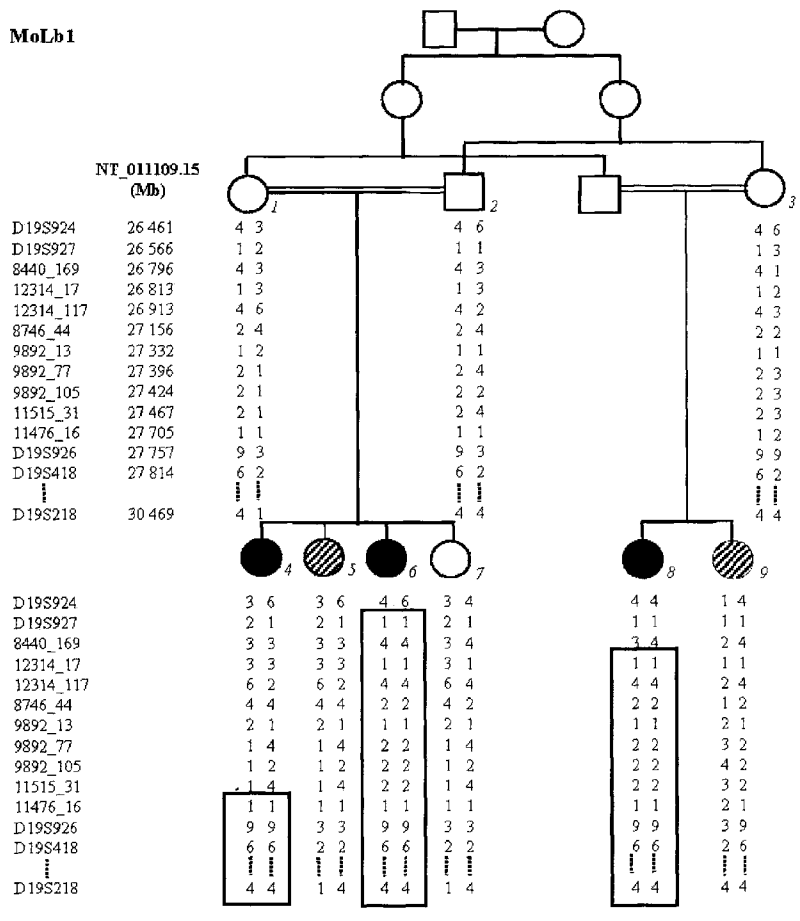
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(54) Title: NALP7-BASED DIAGNOSIS OF FEMALE REPRODUCTIVE CONDITIONS



(57) Abstract: Methods, reagents and kits are described for the diagnosis of a female reproductive condition, based on the detection of an alteration in a NALP7-encoding nucleic acid or a NALP7 polypeptide, relative to a corresponding wild-type NALP7-encoding nucleic acid or NALP7 polypeptide.

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TITLE OF THE INVENTION

[0001] NALP7-BASED DIAGNOSIS OF FEMALE REPRODUCTIVE CONDITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of United States Provisional Patent Application No. 60/704,896 filed August 3, 2005, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to methods and reagents for the diagnosis of conditions of the female reproductive system.

BACKGROUND OF THE INVENTION

[0004] A number of female reproductive conditions exist which not only have adverse effects on fertility, but may pose serious health concerns to female sufferers, such as cancer. Such conditions include gestational trophoblastic diseases, such as the phenomenon of recurrent hydatidiform moles (HM), an abnormal human pregnancy with no embryo and cystic degeneration of placental villi. Recurrent HM is a rare clinical entity in which molar tissues are diploids and have a biparental contribution to their genome. In a number of cases this condition has been observed to have a familial basis. Recurrent hydatidiform molar tissues are undistinguishable at both gross morphology and histopathology levels from the common non-recurrent moles, which are androgenetic in most of the cases (80% of the cases), but may also be biparental (in 20% of the cases). The common form of hydatidiform moles occur in 1 in every 1500 pregnancies in western countries, but at a higher incidence in the Far East, Africa and Central America where the incidence of this condition may reach 1 in 100 pregnancies. Epidemiological studies performed to correlate this higher incidence with various environmental factors failed to reach significant conclusions, but shows a higher risk of hydatidiform moles at the beginning and end of a woman's reproductive cycle. In addition, the relative risk of developing a second HM after a previous molar

pregnancy is 20 to 40 times the incidence of moles in the general population indicating genetic susceptibility to moles.

[0005] In mammals, maternal effect genes, in addition to those coding for oocyte mRNAs and proteins that accumulate in the egg during oogenesis, extend to genes required in the maternal reproductive tract for normal preimplantation and implantation development. Applicant has previously mapped a genetic region responsible for recurrent HMs to a 15-cM interval on 19q13.4 in two unrelated families, MoLb1 and MoGe2 (Moglabey et al., 1999). Additional families from various ethnic groups were reported and most of them were found linked to 19q13.4, indicating a major locus in this region leading to recurrent HMs. The analysis of these families narrowed down the HM candidate region to a 1.1-Mb interval (Sensi *et al.*2000; Hodges *et al.*2003).

[0006] Therefore, there is a continued need to identify the gene associated with such disorders.

[0007] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0008] The invention relates to NALP7 and conditions of the female reproductive system, including diagnosis of such conditions based on NALP7.

[0009] Accordingly, In a first aspect, the invention provides a method for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female (e.g., human) subject, the method comprising detecting an alteration in the sequence of the NALP7 gene or the sequence of its mRNA or encoded polypeptide in a tissue sample from said subject relative to the sequence of the wild-type NALP7 gene or the sequence of its mRNA or encoded polypeptide, wherein said alteration indicates that the subject suffers from or has a predisposition for the reproductive condition.In embodiments, the reproductive condition is selected from gestational trophoplastic disease, gestational trophoblastic tumor, hydatidiform mole, molar

pregnancy, biparental molar pregnancy, androgenetic molar pregnancy, invasive mole, choriocarcinoma, premature ovarian failure, infertility, endometriosis, implantation failure, blighted ovum, recurrent spontaneous abortions, and preeclampsia.

[0010] In embodiments, the alteration is associated with altered splicing of a NALP7 transcript, such as altered splicing of exon 3, exon 7, or both, of said NALP7 gene.

[0011] In an embodiment, the method further comprises amplification of a nucleic acid sequence suspected of comprising the alteration in the sample prior to the detection of the alteration.

[0012] In embodiments, detection of the alteration is performed using a method selected from: (a) sequencing of the NALP7 nucleic acid sequence; (b) hybridization of a nucleic acid probe capable of specifically hybridizing to a NALP7 nucleic acid sequence comprising the alteration and not to a corresponding wild-type NALP7 nucleic acid sequence; (c) restriction fragment length polymorphism analysis (RFLP); (d) amplified fragment length polymorphism PCR (AFLP-PCR); (e) amplification of a nucleic acid fragment comprising a NALP7 nucleic acid sequence using a primer specific for the alteration, wherein the primer produces an amplified product if the alteration is present and does not produce the same amplified product when a corresponding wild-type NALP7 nucleic acid sequence is used as a template for amplification; (f) sequencing of the NALP7 polypeptide; (g) digestion of the NALP7 polypeptide followed by mass spectrometry or HPLC analysis of the peptide fragments, wherein the alteration of the NALP7 polypeptide results in an altered mass spectrometry or HPLC spectrum as compared to wild-type NALP7 polypeptide; and (h) immunodetection using an immunological reagent which exhibits altered immunoreactivity with a NALP7 polypeptide comprising the alteration relative to a corresponding wild-type NALP7 polypeptide.

[0013] In an embodiment, the method further comprises determining cytokine release of an immune cell of said subject, wherein a decrease in cytokine release

relative to a control level of cytokine release is further indicative that the subject suffers from or has a predisposition for the reproductive condition.

[0014] In embodiments, the control level of cytokine release is selected from an established standard and a level of cytokine release of an immune cell comprising a wild-type NALP7 nucleic acid .

[0015] In an embodiment, the method further comprises selecting a prophylactic or therapeutic course of action in accordance with the detected alteration.

[0016] In a further aspect, the invention provides a nucleic acid probe capable of specifically hybridizing to an altered NALP7 nucleotide sequence and not to a corresponding wild-type NALP7 nucleotide sequence.

[0017] The invention further provides a primer or an amplification pair capable of specifically producing an amplified product from a template comprising an altered NALP7 nucleotide sequence and which does not produce the same amplified product from a template comprising a corresponding wild-type NALP7 nucleotide sequence. In embodiments, the primer or amplification pair are selected from SEQ ID NOs: 6-42.

[0018] The invention further provides an isolated altered NALP7 nucleic acid or fragment thereof, wherein said altered NALP7 nucleic acid or fragment thereof comprises a nucleotide sequence comprising an alteration relative to the nucleotide sequence of a wild-type NALP7 nucleic acid or fragment thereof.

[0019] The invention further provides an isolated nucleic acid comprising a sequence that encodes an altered NALP7 polypeptide or fragment thereof.

[0020] The invention further provides an isolated nucleic acid comprising an alteration described herein and which is substantially identical to or substantially complementary to the above-mentioned isolated nucleic acid.

[0021] In an embodiment, the nucleic acid comprises an altered NALP7 nucleotide sequence comprising an alteration associated with altered splicing of a NALP7 transcript, such as altered splicing of exon 3, exon 7, or both, of said NALP7 gene.

[0022] In an embodiment, the alteration occurs at a splice donor site, such as at the splice donor site at the boundary of exon 3 and intron 3, the splice donor site at the boundary of exon 7 and intron 7, or both, of the NALP7 gene.

[0023] In an embodiment, the alteration results in a loss of a cleavage site for a restriction endonuclease (e.g., *Bst*NI) in the NALP7 gene.

[0024] In an embodiment, the alteration is at an amino acid position within the NALP7 polypeptide selected from position 693, 399, 379, 99 and 657 of the NALP7 polypeptide.

[0025] In embodiments, the alteration is selected from a substitution of the C corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide and a substitution of the G corresponding to the second position of the codon for Arg 693 of the NALP7 polypeptide. In further embodiments, the alteration is selected from a substitution of Arg 693 with Trp (R693W).

[0026] In further embodiments, the alteration is selected from (a) a substitution of Cys 399 with Tyr (C399Y); (b) a substitution of Lys 379 with Asn (K379N); (c) a substitution of the codon for Glu 99 with a stop codon (E99X); and (d) a substitution of Asp 657 with Val (D657V).

[0027] In embodiments, the alteration is selected from: (a) a substitution of G with A at the splice donor site at the boundary of exon 3 and intron 3 (IVS3+1G>A); (b) a substitution of G with A at the splice donor site at the boundary of exon 7 and intron 7 (IVS7+1G>A); (c) a substitution of C with T corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide; (d) a substitution of G with A corresponding to the second position of the codon for Cys 84 of the NALP7

polypeptide; (e) a substitution of G with A corresponding to the second position of the codon for Cys 399 of the NALP7 polypeptide; (f) a substitution of G with C corresponding to the third position of the codon for Lys 379 of the NALP7 polypeptide; (g) a substitution of G with T corresponding to the first position of the codon for Glu 99 of the NALP7 polypeptide; and (h) a substitution of A with T corresponding to the second position of Asp 657 of the NALP7 polypeptide

[0028] The invention further provides a replicative cloning vector comprising the above-mentioned nucleic acid and a replicon operative in a host cell.

[0029] The invention further provides a vector (e.g., an expression vector) comprising the above-mentioned nucleic acid operably linked to a transcriptionally regulatory element.

[0030] The invention further provides a host cell transformed with the above-mentioned vector, replicative cloning vector or expression vector.

[0031] The invention further provides an isolated, recombinant or substantially pure altered NALP7 polypeptide encoded by the above-mentioned nucleic acid.

[0032] The invention further provides a polypeptide comprising an alteration described herein and which is substantially identical to the above-mentioned isolated, recombinant or substantially pure altered NALP7 polypeptide.

[0033] The invention further provides an antibody that binds specifically binds the above-mentioned altered NALP7 polypeptide.

[0034] The invention further provides an antibody capable of altered immunoreactivity with a NALP7 polypeptide comprising the alteration relative to a corresponding wild-type NALP7 polypeptide, such as an antibody that selectively binds to the altered NALP7 polypeptide but does not bind to or binds to a lesser extent to a corresponding wild-type NALP7 polypeptide under the same conditions.

[0035] The invention further provides a kit for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female subject, said kit comprising means for detection of an alteration in the sequence of a NALP7 gene or the sequence of its mRNA or encoded polypeptide in a tissue sample from said subject relative to the sequence of a corresponding wild-type NALP7 gene or the sequence of its mRNA or encoded polypeptide. In embodiments, such means are chosen from reagents for: (a) sequencing of the NALP7 nucleic acid sequence; (b) hybridization of a nucleic acid probe capable of specifically hybridizing to a NALP7 nucleic acid sequence comprising the alteration and not to a corresponding wild-type NALP7 nucleic acid sequence; (c) restriction fragment length polymorphism analysis (RFLP); (d) amplified fragment length polymorphism PCR (AFLP-PCR); (e) amplification of a nucleic acid fragment comprising a NALP7 nucleic acid sequence using a primer specific for the alteration, wherein the primer produces an amplified product if the alteration is present and does not produce the same amplified product when a corresponding wild-type NALP7 nucleic acid sequence is used as a template for amplification; (f) sequencing of the NALP7 polypeptide; (g) digestion of the NALP7 polypeptide followed by mass spectrometry or HPLC analysis of the peptide fragments, wherein the alteration of the NALP7 polypeptide results in an altered mass spectrometry or HPLC spectrum as compared to wild-type NALP7 polypeptide; and (h) immunodetection using an immunological reagent which exhibits altered immunoreactivity with a NALP7 polypeptide comprising the alteration relative to a corresponding wild-type NALP7 polypeptide. In embodiments, the reagents are chosen from the above-mentioned antibody, primer (or pair), and probe.

[0036] In an embodiment, the kit further comprises means to determine cytokine release of an immune cell of said subject.

[0037] In an embodiment, the kit further comprises instructions for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female subject

[0038] The Invention further provides a method of identifying a compound for restoring defective immune function associated with a reproductive condition, said method comprising determining whether cytokine release of an immune cell

comprising an altered NALP7 nucleic acid or polypeptide is increased in the presence of a test compound relative to in the absence of said test compound; wherein said increase is indicative that said test compound may be used for for restoring defective immune function associated with a reproductive condition.

[0039] In an embodiment, the immune cell is a a peripheral blood mononuclear cell (PBMC). Iin a further embodiment, the immune cell is a lymphocyte or monocyte.

[0040] In embodiments, the cytokine is selected from interleukin-1β (IL-1 β) and TNF alpha (TNFα).

[0041] Other advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] Figure 1: Partial pedigree of family MoLb1 showing the limit of the proximal boundary of the hydatidiform mole candidate locus on 19q13.4. Markers are ordered (top to bottom) from centromere to telomere and their positions are given in contig NT_011109.15. Genotyping was performed using publicly available and newly generated microsatellite markers by incorporation of radiolabelled nucleotides in the PCR amplification and separation of the products on 5% denaturing polyacrylamide gels. Black symbols indicate affected women, white symbols unaffected, and shaded symbol indicates a woman with unknown disease status. The homozygous region in the three affected sisters is indicated. The black box shows the region that is homozygous in each patient. The proximal border of the candidate region is defined by marker 11515_31 due to its heterozygosity in patient 4.

[0043] Figure 2: Pedigree of family MoPa61 with recurrent hydatidiform moles. Twenty-three informative microsatellite markers were genotyped to determine linkage to 19q13.4. Markers are ordered (top to bottom) from centromere to telomere

and are indicated on the left along with their position in contig NT_011109.15. These data define marker COX6B2 as the distal boundary of the HM candidate region due to its lack of homozygosity in all three affected sisters.

[0044] Figure 3: Segregation of the IVS3+1G>A mutation in family MoLb1. **a,** sequence electropherogram showing the exon3/intron3 boundary in normal control and in patient MoLb1-4. The recognition site of the restriction enzyme *Bst*N1, CCWGG, is abolished by the splice mutation IVS3+1G>A. **b,** Partial pedigree of MoLb1 showing the genotypes of the different members for the IVS3+1 mutation. The band at 206 bp is uncut by *Bst*N1 and thus contains the mutation, the band at 153 bp resulted from the digestion of the normal allele with *Bst*N1. The parents (1, 2, and 3) are heterozygous for the mutation, the affected women, 4, 6, and 8, are homozygous for the mutation, the unaffected sister, 7, is homozygous for the normal allele, as is 5 whose status with respect to molar pregnancy is unknown. Member 9 is a carrier for the mutation and her phenotype is also unknown.

[0045] Figure 4: DNA sequence electrophorograms showing the IVS7+1 G>A, R693W mutations. For each mutation, the control individuals homozygous for the normal alleles are shown at the top; the mothers of the patients who are heterozygous for the normal and mutant alleles are shown in the middle; and affected females, homozygous for the mutations are shown at the bottom. The pedigree symbols are as described in the legend of figure 1.

[0046] Figure 5**:** Abnormal RNA splicing resulting from IVS7+1G>A mutations on RNA extracted from EBV-transformed lymphoblastoid cell lines from the patients. RT PCR using primers located in exons 6 and 8 of *NALP7* in one patient from family MoPa61 amplified a ~ 1kb fragment present only in the patient from MoPa61, but not in a patient from MoLb1 (with IVS+1G>A) or in control. The ZNF28 gene was amplified on the same samples to show the equal amount of cDNA.

[0047] Figure 6: Genomic DNA sequence of human NALP7 (SEQ ID NO: 1; derived from GenBank accession No. NT_011109.15)

[0048] Figure 7: DNA (SEQ ID NO: 2) and polypeptide (SEQ ID NO: 3) sequence of human NALP7, 980 amino acid isoform (GenBank accession No. AY154462 or NM_206828). Coding sequence is defined by position 71-3013 of DNA sequence.

[0049] Figure 8: DNA (SEQ ID NO: 4) and polypeptide (SEQ ID NO: 5) sequence of human NALP7, 1009 amino acid isoform (GenBank accession No. NM_139176). Coding sequence is defined by position 71-3100 of DNA sequence.

[0050] Figure 9: IL-1β secretion by PBMCs with *NALP7* mutations. Blood was collected from one patient from family MoLb1 (Lb1-4 in the right panel) and three patients from MoGe2 (II-2, II-3, II-8, left panel). PBMCs were isolated from blood using the Ficoll gradient technique, 500,000 cells/well were stimulated with 100 ng/mL of LPS, supernatant was collected 20 hours later and IL-1β levels at the indicated dilutions were measured using ELISA.

[0051] Figure 10: TNFα secretion by PBMCs with *NALP7* mutations. TNFα levels were measured as described in Example 8 below .

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0052] In the studies described herein, applicant has identified a defective maternal gene, NALP7, and its causative role in different conditions affecting the female reproductive system, such as recurrent molar pregnancies.

[0053] NALP7 is one of 14 members of the NALP proteins, a large subfamily of the CATERPILLER protein family involved in inflammation and apoptosis. NALP7 is related to the mouse MATER, (also a member of the CATERPILLER protein family). The *NALP7* gene consists of 11 exons encoding for 1009 amino acid protein (the longest isoform). Three transcriptional isoforms *NALP7*V1-V3 involving the alternative splicing of exons 5, 9, and 10 have been described (Okada et al., 2004). NALP7 contains an amino-terminal PYRIN domain (PYD) (also called DAPIN), a putative protein-protein interaction domain found in all the CATERPILLER protein

family and thought to function in apoptotic and inflammatory signaling pathways; a NACHT domain found in neuronal apoptosis inhibitor proteins as well as in those involved in the major histocompatibility complex (MHC) class II transactivation and caspase-recruitment proteins; a nuclear localization signal (NLS) present within the NACHT domain; and 9 to 10 leucine-rich repeats (LRRs) (depending on the splicing isoforms) found in the Ran GTPase activating proteins (RanGAP1), highly conserved proteins essential for nuclear transport, cell cycle regulation, mitotic spindle formation, and post mitotic nuclear envelope assembly. NALP7 has been shown to inhibit caspase-1 dependent IL-1β secretion, which in turn induces NALP7 expression. *NALP7* (also referred to as PYPAF3) was recently shown to be upregulated in testicular seminoma tumors where its down regulation by transfection with small interfering RNA results in growth suppression (Okada et al., 2004; International patent application publication no. WO2004/031410 [Nakamura et al., April 15, 2004]).

[0054] As described herein, applicants have identified a number of mutations in the NALP7 gene in families having female members suffering from reproductive conditions, such as recurrent hydatidiform moles. Such identified mutations include:

a) a substitution of G with A in the GT sequence of the splice donor site at the boundary of exon 3 and intron 3 (IVS3+1G>A) of the NALP7 gene;

b) a substitution of G with A in the GT sequence of the splice donor site at the boundary of exon 7 and intron 7 (IVS7+1G>A) of the NALP7 gene;

c) a substitution of C with T corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide;

d) a substitution of G with A corresponding to the second position of the codon for Cys 84 of the NALP7 polypeptide,

e) a substitution of G with A corresponding to the second position of the codon for Cys 399 of the NALP7 polypeptide,

f) a substitution of G with C corresponding to the third position of the codon for Lys 379 of the NALP7 polypeptide,

g) a substitution of G with T corresponding to the first position of the codon for Glu 99 of the NALP7 polypeptide; and

(h) a substitution of A with T corresponding to the second position of Asp 657 of the NALP7 polypeptide.

[0055] The above mutations (a) and (b) have resulted in incorrect splicing of the NALP7 transcript, notably in respect of the exon 3/intron 3 boundary and the exon 7/intron 7 boundary, respectively. For example of incorrect splicing in case of (b), the mutation was shown to result in the inclusion of the entire intron 7 resulting in the addition of one amino acid (a serine) to exon 7, followed by a stop codon, resulting therefore in a shortened protein of 824 amino acids.

[0056] The above mutation (a) has also resulted in a loss of a cleavage site of the restriction endonuclease *Bst*NI.

[0057] The above mutation (c) has resulted in an alteration at Arg 693 of the NALP7 polypeptide sequence, notably its substitution with Trp. The above mutation (d) has resulted in an alteration at Cys 84 of the NALP7 polypeptide sequence, notably its substitution with Tyr. The above mutation (e) has resulted in an alteration at Cys 399 of the NALP7 polypeptide sequence, notably its substitution with Tyr. The above mutation (f) has resulted in an alteration at Lys 379 of the NALP7 polypeptide sequence, notably its substitution with Asn. The above mutation (g) has resulted in an alteration at Glu 99 of the NALP7 polypeptide sequence, notably its substitution with a stop codon. The above mutation (h) has resulted in an alteration at Asp 657 of the NALP7 polypeptide, notably its substitution with a Val.

[0058] Applicant has further shown herein NALP7 transcription in EBV lymphoblastoid cell lines, normal human uterus, ovaries, unfertilized oocytes at the germinal vesicle and metaphase I stages, early embryo cleavage (1 to 6 cells) and first trimester chorionic villi at 6 and 12 weeks of gestation.

[0059] Accordingly, in an aspect, the invention relates to NALP7-based

diagnosis of conditions of the female reproductive system. The invention thus provides methods and reagents to detect an alteration in NALP7 or its encoded polypeptide, including an alteration in its nucleic acid sequence (including its DNA, mRNA (or cDNA)) or polypeptide sequence, in a sample from a female subject. The presence of an alteration relative to the corresponding wild-type nucleic acid sequence or polypeptide sequence is indicative that the female subject suffers from or has a predisposition for the reproductive condition. The invention further relates to screening to identify compounds capable of restoring defective immune function associated with a female reproductive condition, e.g., that associated with mutant NALP7.

[0060] The invention thus provides a method for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female subject, the method comprising detecting an alteration in the sequence of the NALP7 gene or the sequence of its mRNA or encoded polypeptide in a tissue sample from said subject relative to the sequence of the wild-type NALP7 gene or the sequence of its mRNA or encoded polypeptide. The presence of the alteration indicates that the subject suffers from or has a predisposition for the reproductive condition.

[0061] The invention further provides an *in vitro* method for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female subject, the method comprising detecting an alteration in the sequence of the NALP7 gene or the sequence of its mRNA or encoded polypeptide in a tissue sample from said subject relative to the sequence of the wild-type NALP7 gene or the sequence of its mRNA or encoded polypeptide. The presence of the alteration indicates that the subject suffers from or has a predisposition for the reproductive condition.

[0062] Examples of wild-type NALP7 DNA and polypeptide sequences are provided in Figures 6-8 and SEQ ID NOs 1, 2 and 4 (DNA) and SEQ ID NOs 3 and 5 (polypeptide).

[0063] Applicant has further described herein a decrease in cytokine release in immune cells obtained from a patient harboring a NALP7 mutation. Accordingly, in an

embodiment, the above-mentioned method further comprises determining cytokine release of an immune cell of said subject, wherein a decrease in cytokine release relative to a control level of cytokine release is further indicative that the subject suffers from or has a predisposition for the reproductive condition.

[0064] The above-mentioned control level of cytokine release may be for example an established standard (e.g., a level established in the art for an immune cell capable of wild-type, normal or healthy immune function) or a level of cytokine release of an immune cell comprising a wild-type NALP7 nucleic acid or polypeptide.

[0065] The above-mentioned immune cell may be for example a peripheral blood mononuclear cell (PBMC), lymphocyte, or monocyte.

[0066] In embodiments, the above-mentioned cytokine is selected from interleukin-1β (IL-1 β) and TNF alpha (TNFα).

[0067] In an embodiment, the subject is a female mammal, e.g., a human female subject.

[0068] In embodiments, the reproductive condition is selected gestational trophoplastic disease, gestational trophoblastic tumor, hydatidiform mole, molar pregnancy, biparental molar pregnancy, androgenetic molar pregnancy, invasive mole, choriocarcinoma, premature ovarian failure, infertility, endometriosis, implantation failure, blighted ovum, recurrent spontaneous abortions, preeclampsia, and stillbirth.

[0069] In various embodiments, the above noted tissue sample comprises a tissue or body fluid from the subject, such as blood, serum, lymphocytes, epithelia, endometrial and uterine biopsies, and oocytes.

[0070] "Alteration" as used herein in respect of a nucleotide or polypeptide sequence refers to any type of mutation or change relative to the corresponding wild-type nucleotide or polypeptide sequence, including deletions, insertions, substitutions

and point mutations. In the case of a nucleotide sequence, such an alteration may occur in coding and/or non-coding regions. Mutations of a nucleotide sequence may for example result in the creation of a stop codon, frameshift mutation, altered splicing or an amino acid substitution. In the case of mutations in a regulatory region (e.g., a promoter), a decrease or loss of mRNA expression may result. Accordingly, in various embodiments, the alteration is selected from a deletion from, substitution of and/or insertion into a NALP7 nucleic acid and/or polypeptide sequence.

[0071] In an embodiment, the alteration results in altered splicing relative to wild-type NALP7. Such altered splicing may occur in respect of exon 3 and/or exon 7. In embodiments, the alteration may occur in the splice donor site, such as in the GT splice donor sequence.

[0072] In an embodiment the alteration results in altered sensitivity to a restriction endonuclease, such as a loss of a cleavage site for a restriction endonuclease. In an embodiment, the restriction endonuclease is *Bst*NI.

[0073] In an embodiment, the alteration occurs at position 693 of the NALP7 polypeptide, in further embodiments, the alteration is a substitution of Arg 693 with a different amino acid, such as Trp .

[0074] In an embodiment, the alteration occurs at position Cys 84 of the NALP7 polypeptide, in further embodiments, the alteration is a substitution of Cys 84 with a different amino acid, such as Tyr.

[0075] In an embodiment, the alteration occurs at position 399 of the NALP7 polypeptide, in further embodiments, the alteration is a substitution of Cys 399 with a different amino acid, such as Tyr.

[0076] In an embodiment, the alteration occurs at position 379 of the NALP7 polypeptide, in further embodiments, the alteration is a substitution of Lys 379 with a different amino acid, such as Asn.

[0077] In an embodiment, the alteration occurs at position 99 of the NALP7 polypeptide, in further embodiments, the alteration is a substitution of Glu 99, such as with a stop codon

[0078] In an embodiment, the alteration occurs at position 657 of the NALP7 polypeptide, in further embodiments, the alteration is a substitution of Asp 657 with a different amino acid, such as Val.

[0079] In an embodiment, the alteration occurs at a splice donor and/or splice acceptor site. In an embodiment, the alteration occurs at a splice donor site at the boundary of exon 3 and intron 3, in a further embodiment, at a splice donor site at the boundary of exon 7 and intron 7.

[0080] In further embodiments, the alteration is selected from (a) a substitution of G with A in the GT sequence of the splice donor site at the boundary of exon 3 and intron 3 (IVS3+1G>A) of the NALP7 gene; (b) a substitution of G with A in the GT sequence of the splice donor site at the boundary of exon 7 and intron 7 (IVS7+1G>A) of the NALP7 gene; (c) a substitution of C with T corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide; (d) a substitution of G with A corresponding to the second position of the codon for Cys 84 of the NALP7 polypeptide; (f) a substitution of G with C corresponding to the third position of the codon for Lys 379 of the NALP7 polypeptide; (g) a substitution of G with T corresponding to the first position of the codon for Glu 99 of the NALP7 polypeptide; and (h) a substitution of A with T corresponding to the second position of Asp 657 of the NALP7 polypeptide.

[0081] The above-noted alteration is relative to a wild-type NALP7 sequence, examples of which are provided in Figures 6-8 and SEQ ID NOs 1, 2 and 4 (DNA) and SEQ ID NOs 3 and 5 (polypeptide). The invention further provides an isolated nucleic acid or polypeptide comprising an nucleotide or amino acid sequence selected from SEQ ID NOs 1, 2 and 4 (DNA) and SEQ ID NOs 3 and 5 (polypeptide) further comprising an alteration noted herein or any combination of the alterations noted herein.

[0082] The detection of any combination of the above-noted alterations may also be used in the methods of the invention.

[0083] Further, the above-mentioned method may further comprise selection of a prophylactic or therapeutic course of action in accordance with the detected alteration.

[0084] The above noted alteration may be detected by a number of methods which are known in the art. Examples of suitable methods include sequencing of the NALP7 nucleic acid sequence; hybridization of a nucleic acid probe capable of specifically hybridizing to a NALP7 nucleic acid sequence comprising the alteration and not to (or to a lesser extent to) a corresponding wild-type NALP7 nucleic acid sequence (under comparable hybridization conditions); restriction fragment length polymorphism analysis (RFLP); Amplified fragment length polymorphism PCR (AFLP-PCR); amplification of a nucleic acid fragment comprising a NALP7 nucleic acid sequence using a primer specific for the alteration, wherein the primer produces an amplified product if the alteration is present and does not produce the same amplified product when a corresponding wild-type NALP7 nucleic acid sequence is used as a template for amplification (e.g. allele-specific PCR) ; sequencing of the NALP7 polypeptide; Digestion of the NALP7 polypeptide followed by mass spectrometry or HPLC analysis of the peptide fragments, wherein the alteration of the NALP7 polypeptide results in an altered mass spectrometry or HPLC spectrum as compared to wild-type NALP7 polypeptide; and immunodetection using an immunological reagent (e.g. an antibody, a ligand) which exhibits altered immunoreactivity with a NALP7 polypeptide comprising the alteration relative to a corresponding wild-type NALP7 polypeptide; Immunodetection can measure the amount of binding between a polypeptide molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots, and other techniques known to those of ordinary skill in the art (see Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999 and Edwards R, Immunodiagnostics: A Practical Approach, Oxford University Press,

Oxford; England, 1999). All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., Microarray Biochip Technology, Eaton Publishing, Natick, Mass., 2000).

[0085] Further, NALP7 nucleic acid-containing sequences may be amplified using known methods (e.g. polymerase chain reaction [PCR]) prior to or in conjunction with the detection methods noted herein. Examples of PCR primers for amplification of NALP7 sequences are provided in the Examples herein. The design of various primers for such amplification is known in the art.

[0086] The detection methods herein may also be performed in an assay utilizing a substrate having detection reagents attached thereto at discrete locations, such as a nucleic acid microarray. The invention further provides a substrate comprising an isolated altered NALP7 nucleic acid described herein attached thereto.

[0087] The invention further provides a nucleic acid, e.g., a probe, capable of specifically hybridizing to the altered NALP7 nucleotide sequence and not to (or to a lesser extent to) a corresponding wild-type NALP7 nucleic acid sequence (under comparable hybridization conditions). Such hybridization may be under moderately stringent, or preferably stringent, conditions, e.g. as noted below. Such a probe or plurality thereof may in embodiments be attached to a solid substrate, as noted above.

[0088] The invention further provides (a) nucleic acid primer(s) (e.g. an amplification pair) specific for the alteration, wherein the primer(s) produce(s) an amplified product if the alteration is present and does not produce the same amplified product when a corresponding wild-type NALP7 nucleic acid sequence is used as a template for amplification.

[0089] The invention further provides an isolated nucleic acid encoding the above-mentioned altered NALP7 polypeptide. The invention further provides an isolated altered NALP7 nucleic acid comprising the above noted alteration. The

invention further provides an isolated, substantially pure, or recombinant polypeptide encoded by the above-mentioned nucleic acid, as well as fusion proteins comprising the polypeptide and an additional polypeptide sequence (e.g. a heterologous polypeptide sequence). The invention further provides an isolated, substantially pure, or recombinant polypeptide comprising the above noted alteration. The invention further provides isolated nucleic acids having a nucleotide sequence which is substantially identical to the above-noted altered NALP7 nucleic acid of the invention. The invention further provides an isolated, substantially pure, or recombinant polypeptide having an amino acid sequence which is substantially identical to the above-noted altered NALP7 polypeptide of the invention.

[0090] "Altered NALP7 nucleic acid" or "altered NALP7 gene" as used herein refer to a nucleic acid comprising a nucleotide sequence which differs from a wild-type NALP7 nucleotide sequence in that it comprises an alteration as noted herein. "NALP7 nucleic acid", "NALP7 gene", "wild-type NALP7 nucleic acid" or "wild-type NALP7 gene" as used herein refer to a nucleic acid comprising a nucleotide sequence encoding a NALP7 polypeptide or protein. "NALP7 polypeptide", "NALP7 protein", "wild-type NALP7 polypeptide" or "wild-type NALP7 protein" as used herein refer to a polypeptide comprising the amino acid sequence of a NALP7 polypeptide present in subjects not suffering from a reproductive condition, and having NALP7 activity. Examples of nucleotide sequences of human wild-type NALP7 genes or nucleic acids are set forth in Figures 6-8 and SEQ ID NOs: 1, 2 and 4. Examples of amino acid sequences of human wild-type NALP7 polypeptides or proteins are set forth in Figures 7 and 8 and SEQ ID NOs: 3 (980 amino acid isoform) and 5 (1009 amino acid isoform).

[0091] "Homology" and "homologous" refers to sequence similarity between two peptides or two nucleic acid molecules. Homology can be determined by comparing each position in the aligned sequences. A degree of homology between nucleic acid or between amino acid sequences is a function of the number of identical or matching nucleotides or amino acids at positions shared by the sequences. As the term is used herein, a nucleic acid sequence is "homologous" to another sequence if the two sequences are "substantially identical", as used herein, and the functional activity of the sequences is conserved (as used herein, the term 'homologous' does

not infer evolutionary relatedness). Two nucleic acid sequences are considered "substantially identical" if, when optimally aligned (with gaps permitted), they share at least about 50% sequence similarity or identity, or if the sequences share defined functional motifs. In alternative embodiments, sequence similarity in optimally aligned substantially identical sequences may be at least 60%, 70%, 75%, 80%, 85%, 90% or 95%. As used herein, a given percentage of homology between sequences denotes the degree of sequence identity in optimally aligned sequences. The invention thus further provides a nucleic acid comprising a nucleotide sequence having at least 60%, 70%, 75%, 80%, 85%, 90% or 95% identity with any of SEQ ID Nos 6-42, or with an altered version of any of SEQ ID NOs 1, 2 and 4 (DNA) and SEQ ID NOs 3 and 5 (polypeptide) comprising an alteration noted herein or any combination of the alterations noted herein. An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than about 25 % identity, with any of SEQ ID NOs described herein.

[0092] Substantially complementary nucleic acids are nucleic acids in which the complement of one molecule is "substantially identical" to the other molecule. Two nucleic acid or protein sequences are considered "substantially identical" if, when optimally aligned, they share at least about 70% sequence identity. In alternative embodiments, sequence identity may for example be at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms, such as the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math* 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85: 2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence identity may also be determined using the BLAST algorithm, described in Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software for performing BLAST analysis may be available through the National Center for Biotechnology Information (through the internet at http://www.ncbi.nlm.nih.gov/). The BLAST algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued

threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold. Initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction is halted when the following parameters are met: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program may use as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (Henikoff and Henikoff, 1992, *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10 (or 1 or 0.1 or 0.01 or 0.001 or 0.0001), M=5, N=4, and a comparison of both strands. One measure of the statistical similarity between two sequences using the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. In alternative embodiments of the invention, nucleotide or amino acid sequences are considered substantially identical if the smallest sum probability in a comparison of the test sequences is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0093] An alternative indication that two nucleic acid sequences are substantially complementary is that the two sequences hybridize to each other under moderately stringent, or preferably stringent, conditions. Examples of nucleic acid hybridization conditions are described further below.

[0094] The invention further provides a vector comprising the above-mentioned nucleic acid and a replicon active in a host cell (e.g. replicative cloning vector). The invention further provides a vector comprising the above-mentioned nucleic acid operably-linked to a transcriptionally regulatory sequence (e.g. an expression vector).

[0095] The invention further provides a host cell transformed with the above-

mentioned vector.

[0096] The invention further provides an immunological reagent, such as an antibody, which exhibits different immunoreactivity with an altered NALP7 polypeptide, i.e., comprising the above-noted alteration, relative to a wild-type NALP7 polypeptide.

[0097] As noted above, an isolated nucleic acid, for example a nucleic acid sequence encoding a polypeptide of the invention, or homolog, fragment or variant thereof, may further be incorporated into a vector, such as a recombinant expression vector. In an embodiment, the vector will comprise transcriptional regulatory sequences or a promoter operably–linked to a nucleic acid comprising a sequence capable of encoding a peptide compound, polypeptide or domain of the invention. A first nucleic acid sequence is "operably-linked" with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably-linked to a coding sequence if the promoter affects the transcription or expression of the coding sequences. Generally, operably-linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in reading frame. However, since for example enhancers generally function when separated from the promoters by several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably-linked but not contiguous. "Transcriptional regulatory sequence/element" is a generic term that refers to DNA sequences, such as initiation and termination signals, enhancers, and promoters, splicing signals, polyadenylation signals which induce or control transcription of protein coding sequences with which they are operably-linked. "Promoter" refers to a DNA regulatory region capable of binding directly or indirectly to RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of the present invention, the promoter is bound at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter will be found a transcription initiation site (conveniently defined by mapping with S1 nuclease), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but

not always, contain "TATA" boses and "CCAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

[0098] As used herein, "nucleic acid molecule", refers to a polymer of nucleotides. Non-limiting examples thereof include DNA (i.e. genomic DNA, cDNA) and RNA molecules (i.e. mRNA). The nucleic acid molecule can be obtained by cloning techniques or synthesized. DNA can be double-stranded or single-stranded (coding strand or non-coding strand [antisense]).

[0099] The term "recombinant DNA" as known in the art refers to a DNA molecule resulting from the joining of DNA segments. This is often referred to as genetic engineering.

[00100] The terminology "amplification pair" refers herein to a pair of oligonucleotides (oligos) of the present invention, which are selected to be used together in amplifying a selected nucleic acid sequence by one of a number of types of amplification processes, preferably a polymerase chain reaction. Other types of amplification processes include ligase chain reaction, strand displacement amplification, or nucleic acid sequence-based amplification. As commonly known in the art, the oligos are designed to bind to a complementary sequence under selected conditions. Accordingly, the invention further provides an amplification pair capable of amplifying an altered NALP7 nucleic acid, a wild-type NALP7 nucleic acid, or a fragment of an altered NALP7 nucleic acid or a wild-type NALP7 nucleic acid. Examples of suitable amplification pairs are set forth in Example 6 below, whereby any suitable combination of forward (fwd) and reverse (rev) primers for a given region are shown (both those utilized for PCR and sequencing may be used as an amplification pair). For example: For Exon 1, representative amplification pairs include SEQ ID NOs: 6 and 7, and SEQ ID NOs: 6 and 35. For Exon 2, representative amplification pairs include SEQ ID NOs: 8 and 9, and SEQ ID NOs: 8 and 36. For Exon 3, representative amplification pairs include SEQ ID NOs: 10 and 11, and SEQ ID NOs: 10 and 37. For Exon 4, representative amplification pairs include SEQ ID NOs: 12 and 13, SEQ ID NOs: 14 and 15, SEQ ID NOs: 16 and 17, and SEQ ID NOs: 18 and 19. For Exon 5, representative amplification pairs include SEQ ID NOs: 20 and 21, and SEQ ID NOs: 20 and 38. For Exon 6, a representative

amplification pair is SEQ ID NOs: 22 and 23. For Exon 7, representative amplification pairs include SEQ ID NOs: 24 and 25, and SEQ ID NOs: 39 and 25. For Exon 8, representative amplification pairs include SEQ ID NOs: 26 and 27, and SEQ ID NOs: 41 and 42. For Exon 9, a representative amplification pair is SEQ ID NOs: 28 and 29. For Exon 10, representative amplification pairs include SEQ ID NOs: 30 and 31, and SEQ ID NOs: 30 and 40. For Exon 11, a representative amplification pair is SEQ ID NOs: 32 and 33. For the region comprising the IVS3+1 G>A mutation described herein, a representative amplification pair is SEQ ID NOs: 10 and 34.

[00101] Oligonucleotide probes or primers of the present invention may be of any suitable length, depending on the particular assay format and the particular needs and targeted sequences employed. In general, the oligonucleotide probes or primers are at least 12 nucleotides in length, preferably between 15 and 24 molecules, and they may be adapted to be especially suited to a chosen nucleic acid amplification system. As commonly known in the art, the oligonucleotide probes and primers can be designed by taking into consideration the melting point of hybridization thereof with its targeted sequence (see below and in Sambrook et al., 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, CSH Laboratories; Ausubel et al., 1989, in Current Protocols in Molecular Biology, John Wiley & Sons Inc., N.Y.).

[00102] "Nucleic acid hybridization" refers generally to the hybridization of two single-stranded nucleic acid molecules having complementary base sequences, which under appropriate conditions will form a thermodynamically favored double-stranded structure. Examples of hybridization conditions can be found in the two laboratory manuals referred above (Sambrook et al., 1989, *supra* and Ausubel, *et al.* (eds), 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York,) and are commonly known in the art. Hybridization to filter-bound sequences under moderately stringent conditions may, for example, be performed in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.2 x SSC/0.1% SDS at 42°C (see Ausubel, *et al.* (eds), 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Alternatively, hybridization to filter-bound sequences under stringent conditions may, for example, be performed in 0.5 M $NaHPO_4$, 7% SDS, 1 mM EDTA at 65°C, and

washing in 0.1 x SSC/0.1% SDS at 68°C (see Ausubel, *et al.* (eds), 1989, *supra*). In other examples of hybridization, a nitrocellulose filter can be incubated overnight at 65°C with a labeled probe in a solution containing 50% formamide, high salt (5 x SSC or 5 x SSPE), 5 x Denhardt's solution, 1% SDS, and 100 µg/ml denatured carrier DNA (i.e. salmon sperm DNA). The non-specifically binding probe can then be washed off the filter by several washes in 0.2 x SSC/0.1% SDS at a temperature which is selected in view of the desired stringency: room temperature (low stringency), 42°C (moderate stringency) or 65°C (high stringency). Hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, *Laboratory Techniques in Biochemistry and Molecular Biology -- Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York). The selected temperature is based on the melting temperature (Tm) of the DNA hybrid (Sambrook et al. 1989, *supra*). Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point for the specific sequence at a defined ionic strength and pH. Of course, RNA-DNA hybrids can also be formed and detected. In such cases, the conditions of hybridization and washing can be adapted according to well-known methods by the person of ordinary skill. Stringent conditions will be preferably used (Sambrook et al.,1989, *supra*).

[00103] Probes or primers of the invention can be utilized with naturally occurring sugar-phosphate backbones as well as modified backbones including phosphorothioates, dithionates, alkyl phosphonates and α-nucleotides and the like. Modified sugar-phosphate backbones are generally taught by Miller, 1988, Ann. Reports Med. Chem. 23:295 and Moran et al., 1987, Nucleic acid molecule. Acids Res., 14:5019. Probes or primers of the invention can be constructed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), and preferably of DNA.

[00104] The types of detection methods in which probes can be used include Southern blots (DNA detection), dot or slot blots (DNA, RNA), and Northern blots (RNA detection). Although less preferred, labeled proteins could also be used to detect a particular nucleic acid sequence to which it binds.

[00105] Although the present invention is not specifically dependent on the use of a label for the detection of a particular nucleic acid sequence, such a label might be beneficial, by increasing the sensitivity of the detection. Furthermore, it enables automation (the same can also be said of detection of proteins using ligands such as antibodies). Probes can be labeled according to numerous well-known methods (Sambrook et al., 1989, supra). Non-limiting examples of detectable markers include ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radionucleotides. It will be understood by the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe.

[00106] As commonly known, radioactive nucleotides can be incorporated into probes of the invention by several methods. Non-limiting examples thereof include kinasing the 5' ends of the probes using gamma ^{32}P ATP and polynucleotide kinase, using the Klenow fragment of Pol I of *E. coli* in the presence of radioactive dNTP (e.g. uniformly labeled DNA probe using random oligonucleotide primers in low-melt gels), using the SP6/T7 system to transcribe a DNA segment in the presence of one or more radioactive NTP, and the like.

[00107] As used herein, "oligonucleotides" or "oligos" define a molecule having two or more nucleotides (ribo or deoxyribonucleotides). The size of the oligo will be dictated by the particular situation and ultimately on the particular use thereof and adapted accordingly by the person of ordinary skill. An oligonucleotide can be synthesized chemically or derived by cloning according to well-known methods.

[00108] As used herein, a "primer" defines an oligonucleotide which is capable of annealing to a target sequence, thereby creating a double stranded region which can serve as an initiation point for DNA synthesis under suitable conditions.

[00109] Amplification of a selected, or target, nucleic acid sequence may be carried out by a number of suitable methods. See generally Kwoh et al., 1990, Am. Biotechnol. Lab. 8:14-25. Numerous amplification techniques have been described

and can be readily adapted to suit particular needs of a person of ordinary skill. Non-limiting examples of amplification techniques include polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), transcription-based amplification, the Qβ replicase system and NASBA (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86, 1173-1177; Lizardi et al., 1988, BioTechnology 6:1197-1202; Malek et al., 1994, Methods Mol. Biol., 28:253-260; and Sambrook et al., 1989, *supra*). Preferably, amplification will be carried out using PCR.

[00110] Polymerase chain reaction (PCR) is carried out in accordance with known techniques. See, e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; and 4,965,188 (the disclosures of all three U.S. Patent are incorporated herein by reference). In general, PCR involves, a treatment of a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) under hybridizing conditions, with one oligonucleotide primer for each strand of the specific sequence to be detected. An extension product of each primer which is synthesized is complementary to each of the two nucleic acid strands, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample is analyzed to assess whether the sequence or sequences to be detected are present. Detection of the amplified sequence may be carried out by visualization following EtBr staining of the DNA following gel electrophoresis, or using a detectable label in accordance with known techniques, and the like. For a review on PCR techniques (see PCR Protocols, A Guide to Methods and Amplifications, Michael et al. Eds, Acad. Press, 1990).

[00111] Ligase chain reaction (LCR) is carried out in accordance with known techniques (Weiss, 1991, Science 254:1292). Adaptation of the protocol to meet the desired needs can be carried out by a person of ordinary skill. Strand displacement amplification (SDA) is also carried out in accordance with known techniques or adaptations thereof to meet the particular needs (Walker et al., 1992, Proc. Natl. Acad. Sci. USA 89:392-396; and ibid., 1992, Nucleic Acids Res. 20:1691-1696).

[00112] The term "vector" is commonly known in the art and defines a plasmid

DNA, phage DNA, viral DNA and the like, which can serve as a DNA vehicle into which DNA of the present invention can be cloned. Numerous types of vectors exist and are well known in the art.

[00113] The term "expression" defines the process by which a gene is transcribed into mRNA (transcription), the mRNA is then being translated (translation) into one polypeptide (or protein) or more.

[00114] The recombinant expression vector of the present invention can be constructed by standard techniques known to one of ordinary skill in the art and found, for example, in Sambrook et al. (*supra*). A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments and can be readily determined by persons skilled in the art. The vectors of the present invention may also contain other sequence elements to facilitate vector propagation (e.g. a replicon) and selection in bacteria and host cells. In addition, the vectors of the present invention may comprise a sequence of nucleotides for one or more restriction endonuclease sites. Coding sequences such as for selectable markers and reporter genes are well known to persons skilled in the art.

[00115] A recombinant expression vector comprising a nucleic acid sequence of the present invention may be introduced into a host cell, which may include a living cell capable of expressing the protein coding region from the defined recombinant expression vector. The living cell may include both a cultured cell and a cell within a living organism. Accordingly, the invention also provides host cells containing the recombinant expression vectors of the invention. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00116] Vector DNA can be introduced into cells via conventional

transformation or transfection techniques. The terms "transformation" and "transfection" refer to techniques for introducing foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can for example be found in Sambrook et al. (*supra*), and other laboratory manuals.

[00117] Recombinant production is useful for the preparation of large quantities of the protein encoded by the DNA sequence of interest. The protein can be purified according to standard protocols that take advantage of the intrinsic properties thereof, such as size and charge (i.e. SDS gel electrophoresis, gel filtration, centrifugation, ion exchange chromatography...). In addition, the protein of interest can be purified via affinity chromatography using polyclonal or monoclonal antibodies or other affinity-based systems (e.g. using a suitable incorporated "tag" in the form of a fusion protein and its corresponding ligand). Suitable recombinant systems include prokaryotic and eukaryotic expression systems, which are known in the art.

[00118] The term "allele" defines an alternative form of a gene which occupies a given locus on a chromosome.

[00119] As commonly known, a "mutation" is a detectable change in the genetic material which can be transmitted to a daughter cell. As well known, a mutation can be, for example, a detectable change in one or more deoxyribonucleotide. For example, nucleotides can be added, deleted, substituted for, inverted, or transposed to a new position. Spontaneous mutations and experimentally induced mutations exist. A mutant polypeptide can be encoded from a mutant nucleic acid molecule. In addition, mutant proteins can be produced through aberrant events during replication, transcription and/or translation. Frameshifting (the switching from a particular reading frame to another) is such a mechanism that can modify the sequence of the translated protein.

[00120] A compound is "substantially pure" when it is separated from the components that naturally accompany it. Typically, a compound is substantially pure

when it is at least 60%, more generally 75% or over 90%, by weight, of the total material in a sample. Thus, for example, a polypeptide that is chemically synthesized or produced by recombinant technology will generally be substantially free from its naturally associated components. A nucleic acid molecule is substantially pure when it is not immediately contiguous with (i.e., covalently linked to) the coding sequences with which it is normally contiguous in the naturally occurring genome of the organism from which the DNA of the invention is derived. A substantially pure compound can be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid molecule encoding a polypeptide compound; or by chemical synthesis. Purity can be measured using any appropriate method such as column chromatography, gel electrophoresis, HPLC, etc.

[00121] As used herein, the terms "molecule", "compound", "agent", or "ligand" are used interchangeably and broadly to refer to natural, synthetic or semi-synthetic molecules or compounds. The term "molecule" therefore denotes for example chemicals, macromolecules, cell or tissue extracts (from plants or animals) and the like. Non-limiting examples of molecules include nucleic acid molecules, peptides, antibodies, carbohydrates and pharmaceutical agents. The agents can be selected and screened by a variety of means including random screening, rational selection and by rational design using for example protein or ligand modelling methods such as computer modelling.

[00122] A further aspect of the invention provides an antibody that recognizes an altered NALP7 polypeptide of the invention. Antibodies may be recombinant, *e.g.*, chimeric (*e.g.*, constituted by a variable region of murine origin associated with a human constant region), humanized (a human immunoglobulin constant backbone together with hypervariable region of animal, *e.g.*, murine, origin), and/or single chain. Both polyclonal and monoclonal antibodies may also be in the form of immunoglobulin fragments, *e.g.*, $F(ab)'_2$, Fab or Fab' fragments. The antibodies of the invention are of any isotype, *e.g.*, IgG or IgA, and polyclonal antibodies are of a single isotype or a mixture of isotypes. In general, techniques for preparing antibodies (including monoclonal antibodies and hybridomas) and for detecting antigens using antibodies are well known in the art.

[00123] Antibodies against the altered NALP7 polypeptide of the present invention are generated by immunization of a mammal with a partially purified fraction comprising altered NALP7 polypeptide. Such antibodies may be polyclonal or monoclonal. Methods to produce polyclonal or monoclonal antibodies are well known in the art. For a review, see Harlow and Lane (1988) and Yelton *et al.* (1981), both of which are herein incorporated by reference. For monoclonal antibodies, see Kohler and Milstein (1975), and Campbell, 1984, In "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", Elsevier Science Publisher, Amsterdam, The Netherlands.

[00124] The antibodies of the invention, which are raised to a partially purified fraction comprising altered NALP7 polypeptide of the invention, are produced and identified using standard immunological assays, *e.g.*, Western blot analysis, dot blot assay, or ELISA (see, *e.g.*, Coligan *et al.* (1994), herein incorporated by reference). The antibodies are used in diagnostic methods to detect the presence of a altered NALP7 polypeptide and activity in a sample, such as a tissue or body fluid. The antibodies are also used in affinity chromatography for obtaining a purified fraction comprising the altered NALP7 polypeptide and activity of the invention.

[00125] Accordingly, a further aspect of the invention provides (i) a reagent for detecting the presence of altered NALP7 polypeptide and activity in a tissue or body fluid; and (ii) a diagnostic method for detecting the presence of altered NALP7 polypeptide and activity in a tissue or body fluid, by contacting the tissue or body fluid with an antibody of the invention, such that an immune complex is formed, and by detecting such complex to indicate the presence of altered NALP7 polypeptide and activity in the sample or the organism from which the sample is derived.

[00126] Those skilled in the art will readily understand that the immune complex is formed between a component of the sample and the antibody, and that any unbound material is removed prior to detecting the complex. It is understood that an antibody of the invention is used for screening a sample, such as, for example, blood, plasma, lymphocytes, cerebrospinal fluid, urine, saliva, epithelia and fibroblasts, for the presence of an altered NALP7 polypeptide.

[00127] For diagnostic applications, the reagent (*i.e.*, the antibody of the invention) is either in a free state or immobilized on a solid support, such as a tube, a bead, or any other conventional support used in the field. Immobilization is achieved using direct or indirect means. Direct means include passive adsorption (non-covalent binding) or covalent binding between the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with a reagent is first attached to the solid support. Indirect means may also employ a ligand-receptor system, for example, where a molecule such as a vitamin is grafted onto the reagent and the corresponding receptor immobilized on the solid phase. This is illustrated by the biotin-streptavidin system. Alternatively, a peptide tail is added chemically or by genetic engineering to the reagent and the grafted or fused product immobilized by passive adsorption or covalent linkage of the peptide tail.

[00128] The present invention also relates to a kit for diagnosing a condition of the female reproductive system, or a predisposition to contracting same, comprising suitable means to detect the above-mentioned alteration, such as a probe, primer (or primer pair), or immunological reagent (e.g. antibody) in accordance with the present invention. For example, a compartmentalized kit in accordance with the present invention includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers may for example include a container which will accept the test sample (DNA, protein or cells), a container which contains the primers used in the assay, containers which contain enzymes, containers which contain wash reagents, and containers which contain the reagents used to detect the indicator products. In an embodiment the kit further comprises instructions for diagnosing a condition of the female reproductive system, or a predisposition to contracting same.

[00129] In another aspect, the invention relates to the use of a NALP7-defective immune cell (e.g., having a mutated [e.g., comprising an alteration described herein] or disrupted NALP7 gene, lacking a NALP7 gene, or having been

treated or engineered for decreased NALP7 expression or function [e.g., via NALP7-targeted RNA interference or antisense oligonucleotides]) in screening assays that may be used to identify compounds that are capable of restoring defective immune function associated with a female reproductive condition noted herein. In some embodiments, such an assay may comprise the steps of (a) providing a test compound; (b) providing a a NALP7-defective immune cell; and (c) determining cytokine release in the presence versus the absence of the test compound. An increase in cytokine release in the presence versus the absence of the compound is indicative that the compound is capable of restoring defective immune function associated with a female reproductive condition.

[00130] The above-mentioned immune cell may be for example a peripheral blood mononuclear cell (PBMC), lymphocyte or monocyte. The above-mentioned cytokine may be for example interleukin-1β (IL-1 β) or TNF alpha (TNFα).

[00131] Cytokine release may in embodiments be measured in response to a suitable stimulus, such as in response to bacterial lipopolysaccharide (LPS) as described in the Examples below.

[00132] The above-noted assays may be applied to a single test compound or to a plurality or "library" of such compounds (e.g. a combinatorial library). Any such compounds may be utilized as lead compounds and further modified to improve their therapeutic, prophylactic and/or pharmacological properties.

[00133] Such assay systems may comprise a variety of means to enable and optimize useful assay conditions. Such means may include but are not limited to: suitable buffer solutions, for example, for the control of pH and ionic strength and to provide any necessary components for optimal stability (e.g. protease inhibitors) of assay components, temperature control means for optimal activity and or stability of assay components, and detection means to enable the detection of the indicator product. A variety of such detection means may be used, including but not limited to one or a combination of the following: radiolabelling (e.g. ^{32}P, ^{14}C, ^{3}H), antibody-based detection, fluorescence, chemiluminescence, spectroscopic methods (e.g.

generation of a product with altered spectroscopic properties), various reporter enzymes or proteins (e.g. horseradish peroxidase, green fluorescent protein), specific binding reagents (e.g. biotin/(streptavidin)), and others.

[00134] The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLES

[00135] **Example 1:** Methods

[00136] *Mutation screening and analysis.* Genomic structure of the screened genes were obtained from publicly available databases (http://genome.ucsc.edu/) and the primers flanking predicted exons, exon/intron boundaries and 5'and 3'UTRs were designed using Primer Select v5.05 (DNAStar). Exons were PCR amplified, visualized on 2% agarose gels stained with ethidium bromide, and sequenced directly using a 3730XL DNA Analysis System (Applied Biosystems). Sequences were aligned using SeqManII v5.05 and screened for mutations.

[00137] *RT-PCR.* Total RNAs was extracted from EBV transformed lymphoblast cell lines using Trizol (Invitrogen). Three micrograms of total RNA were reverse-transcribed using 200 units of M-MLV Reverse Transcriptase (Invitrogen) with RNA Guard RNase Inhibitor (Amersham) in a total volume of 50µl. Five microliters of this preparation were then PCR amplified according to standard protocols. Sequencing of cDNA fragments were done on direct PCR products or after purification of the appropriate bands and cloning using the TOPO TA™ Cloning Kit (Invitrogen).

[00138] **Example 2:** Fine mapping of the HM candidate region

[00139] To identify the defective gene associated with recurrent HMs, applicant screened all the predicted exons of the 53 genes present in the reported hypothetical 1.1-Mb minimal interval (Sensi *et al.*; Hodges *et al.*). However, applicant did not find

any mutations in this region. Applicant thus confirmed that the proximal boundary of the reported 1.1-Mb minimal interval is incorrect. Using a proximal boundary identified in family MoLb1 as a 1.29-Mb region between D19S924 and D19S926 (Moglabey et al., 1999), applicant identified herein nine new polymorphic markers from the available genomic DNA sequences and genotyped them in MoLb1. This analysis defined marker 11515-31 as the proximal boundary of the HM candidate region (Figure 1). This new definition of the proximal boundary added a cluster of killer-cell immunoglobulin-like receptors (KIR) genes (7 to 14 genes depending on haplotypes), two KIR-related genes, NCR1, and FCAR, and NALP7. Genotyping of an additional family, MoPa61 previously reported by Mazhar and Janjua (1995) with 23 polymorphic markers from 19q13.4 demonstrated its linkage to this region and defined a single nucleotide polymorphism (SNP) located 16 bases upstream exon 3 of gene COX6B2 (NM_144613) as the distal boundary of the minimal HM candidate region (Figure 2). Based on data from MoLb1 and MoPa61, applicant fine mapped the HM candidate region to 0.65-Mb between 11515-31 and COX6B2ex3.

[00140] **Example 3**: Mutation analyses

[00141] By screening the additional genes identified by the new definition of the proximal boundary herein, applicant identified in *NALP7* (also called *PYPAF3*) two different mutations affecting the invariant G of the GT splice donor site at the junction of exon 3/intron 3 (IVS3+1 G>A) in a patient from MoLb1 (Figure 3a) and at the junction of exon 7/intron 7 (IVS7+1 G>A) in a patient from MoPa61 (Figure 4). The mutation in family MoLb1 abolishes a recognition site for the restriction enzyme *Bst*NI that applicant used to detect the mutation in the other members of the family (Figure 3b) and in 100 control women (with 5 to 16 children) from various ethnic groups. In family MoPa61, IVS7+1G>A, the mutation was investigated in the other members of the family and controls by DNA sequencing (Figure 4). Both mutations segregate with the disease phenotype in their respective families and were not found in the 200 control chromosomes screened. In family MoGe2, applicant identified in exon 5, a C to T change substituting an arginine for a tryptophan at amino acid 693, R693W (Figure 4), a conserved residue in chimpanzee and cow *NALP7* as well as in human, cow, and dog *NALP2*. By DNA sequencing, it was found that this change co-segregates with the disease status in MoGe2 and is not present on 274

chromosomes from control women with five to sixteen children.

[00142] To assess the role of *NALP7* in recurrent molar pregnancies occurring in single-family members that are not homozygous at 19q13.4 markers and could not be investigated for linkage to 19q13.4 (because of the absence of other female siblings with known pregnancy outcomes in the family), applicant screened *NALP7* in eight such cases and identified additional set of five new mutations, C399Y, E99X, C84Y, K379N, and D657V that were not found in controls. Mutations, clinical data, and coding DNA polymorphisms found in the different families and patients are summarized in Table 1.

Table 1. Summary of mutations, ethnic origin, and clinical manifestations of the patients

Family	Population	Location	Nucleotide change	Amino acid change	Clinical manifestations and outcomes	Reference
Familial cases of recurrent moles						
MoLb1	Lebanese	Intron 3	IVS3+1G>A		NP, SB, SA, CHM, PHM, PTD, preeclampsia	Seoud et al, 1995, Helwani et al., 1999
MoPa61	Pakistani	Intron 7	IVS7+1G>A		SA, CHM	Mazhar and Janjua 1995
MoGe2	German	Exon 5	2077C>T	R693W	CHM	Kircheisen and Ried, 1994
MoCh76	Chinese	Exon 3 Exon 5	365G>T 2040A>T	E99X D657V	SB, CHM	Present study
Single family member with recurrent moles						
MoCh71	Chinese	Exon 2	321G>A	C84Y Heterozygous	2 CHMs	Present study
MoCh73	Chinese	Exon 4	1207G>C	K379N Heterozygous	2 CHMs, 1 PHM	Present study
MoCa57	Moroccan	Exon 4	1266G>A	C399Y Heterozygous	SA, BO, TP + CHM	Present study

With reference to Table 1, the phenotype of the conceptuses were as reported in the original papers listed under Reference. Nucleotide positions are given according to RefSeq mRNA NM_206828, amino acid positions according to Q8WX94. NP, normal pregnancy; SB, stillbirth; SA, spontaneous abortion; CHM, complete hydatidiform mole, PHM, partial hydatidiform mole PTD, persistent trophoblastic disease; BO, blighted ovum; TP, twin pregnancy.

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[00143] **Example 4:** Expression of *NALP7* in normal tissues

[00144] A recent study has reported *NALP7* expression in a broad range of normal adult tissues (Kinoshita et al., 2005). To investigate the role of *NALP7* in the pathology of moles, a disease caused by a maternal defective gene, applicant investigated its transcription by RT-PCR in normal human uterus and ovary using two combinations of primers located in exons 6 and 8, and in exons 8 and 11. Applicant identified two *NALP7* transcripts, V1 and V2, in both tissues and also in EBV lymphoblastoid cell lines from normal subjects, and first trimester chorionic villi. By DNA sequencing, we found that V1 and V2 are due to the exclusion or inclusion of exon 10, respectively.

[00145] **Example 5:** Effect of the splice mutations on NALP7 transcription

[00146] The two splice mutations identified herein affect exons 3 and 7 (these exons are present in all reported transcriptional isoforms). Using GENSCAN (http://genes.mit.edu/GENSCAN.html), the splice mutation IVS3+1G>A was predicted to result in the skipping of exon 3, while SSPNN (http://www.fruitfly.org/seq_tools/splice.html) analysis, predicts the activation and usage of a cryptic intronic splice site located 4-bp downstream of exon 3. Using both programs, GENSCAN and SSPNN, the splice mutation IVS7+1 G>A is predicted to lead to the skipping of exon 7. Primers located in exons 6 and 8 amplified a large fragment (~1 kb) in the three patients from MoPa61, that does not correspond to the size of the genomic fragment (2635 bp) between the two primers (Figure 5). This fragment was observed only after reverse transcription and was not present in 5 normal control subjects. Applicant cloned and sequenced this fragment and found it to correspond to the inclusion of the entire intron 7. The inclusion of intron 7 is expected to add next to exon 7 only one amino acid, a serine, followed by a stop codon, TAA, leading to a shorter protein of 824 amino acids.

[00147] **Example 6:** Primers for PCR amplification of regions of NALP7:

Exon 1:

PCR

Fwd: NALP7ex1a GCCCAATTACAGCCAAATCCCTGAG (SEQ ID NO: 6)

Rev: NALP7ex1b GGCCGAGGCAGACAGATTACCTAAA (SEQ ID NO: 7)

Product Size: 604bp

Sequencing

NALP7ex1a (see SEQ ID NO: 6 above)

NALP7Rev2 TCCTTCCAGCATCCTCGCAC (SEQ ID NO: 35)

Exon 2:

PCR

NALP7ex2-fwd ACCGTGCTGGGCCAGATTTTCAGT (SEQ ID NO: 8)

NALPex3-rev GCAGAGGTTGCAATGAGCAGAGACG (SEQ ID NOs: 9; 11)

Product size: 777bp

Sequencing

NALP7ex2-fwd (see SEQ ID NO: 8 above)

NALP7ex2rev2 ATGACCAGGACACCCCAGGTTCTA (SEQ ID NO: 36)

Exon3:

PCR

NALPex3-fwd CCACCATGCCTGGCTGACACTTTAT (SEQ ID NO: 10)

NALPex3-rev GCAGAGGTTGCAATGAGCAGAGACG (SEQ ID NOs: 11; 9)

Product size: 340bp

Sequencing

NALP7ex3-fwd (see SEQ ID NO: 10 above)

NALP3ex2rev2 CACCTTGCATGCTCTCAAACACCA (SEQ ID NO: 37)

Exon 4:

1 -

PCR

NALP7ex4-1 fwd GTAGTGGCTCCGTCTCTGCTCATTG (SEQ ID NO: 12)

NALP7ex4-1 rev AGGCCATCGACCACGAACAGGATTC (SEQ ID NO: 13)

Product Size: 737bp

Sequencing

NALP7ex4-1 fwd (see SEQ ID NO: 12 above)

NALP7ex4-1 rev (see SEQ ID NO: 13 above)

2 –

PCR

NALPex4- 2 fwd GACGACGTCACTCTGAGAAACCAAC (SEQ ID NO: 14)

NALPex4- 2 rev TGCAGAGGAAACGCAGGAACAGC (SEQ ID NO: 15)

Product size: 757bp

Sequencing

NALPex4- 2 fwd (see SEQ ID NO: 14 above)

NALPex4- 2 rev (see SEQ ID NO: 15 above)

3 –

PCR

NALP7ex4-3 fwd TTTGCTGAAGAGGAAGATGTTACCC (SEQ ID NO: 16)

NALP7ex4-3 rev CGAGGCCGAATAAGAAGTGTCCTAC (SEQ ID NO: 17)

Product size: 722bp

Sequencing

NALPe7x4-3 fwd (see SEQ ID NO: 16 above)

NALP7ex4-3 rev (see SEQ ID NO: 17 above)

4 –

PCR

NALP7ex4-4 fwd GTGGGCGCAGATGTCCGTGTTC (SEQ ID NO: 18)

NALP7ex4-4 rev CCTAATTGCCAAGTCGTGTCTCC (SEQ ID NO: 19)

Product size: 803bp

Sequencing

NALP7ex4-4 fwd (see SEQ ID NO: 18 above)

NALP7ex4-4 rev (see SEQ ID NO: 19 above)

Exon 5:

PCR

NALP7ex-5 fwd GGTCTCAGTTTCTAGCCCAAGTT (SEQ ID NO: 20)

NALP7ex-5 rev ACACGGTGAAAACCTGTCTGTGC (SEQ ID NO: 21)

Sequencing

NALP7ex-5 fwd (see SEQ ID NO: 20 above)

NALP7ex5rev2_Seq CAAGAAGCTTAGTCATCGTT (SEQ ID NO: 38)

Product size: 839bp

Exon 6:

PCR

NALP7ex6-fwd CCACTGCACCCGGCCAAGAACTT (SEQ ID NO: 22)

NALP7ex6-rev GCTGGGGGCCACTGCTCTCAATC (SEQ ID NO: 23)

Product size: 597bp

Sequencing

NALP7ex6-fwd (see SEQ ID NO: 22 above)

NALP7ex6-rev (see SEQ ID NO: 23 above)

Exon 7:

PCR

NALP7ex7-fwd GATCACGCCTTTGCATTCCAGACTG (SEQ ID NO: 24)

NALP7ex7-rev AACTCAGATGATCCGCCCACCTCTC (SEQ ID NO: 25)

Product size: 471bp

Sequencing

NALP7ex7Seq AGCTGATAGGGTATACTCTG (SEQ ID NO: 39)

NALP7ex7-rev (see SEQ ID NO: 25 above)

Exon 8:

PCR

NALP7ex8 fwd AAAACAACACCTGTGTCCTGTGATG (SEQ ID NO: 26)

NALP7ex8 rev TTAACATGTTTCTACCTGTATCTGC (SEQ ID NO: 27)

Product size: 849bp

NALP7ex8f2 TGGCCATGATGACTCCCACAGG (SEQ ID NO: 41)

NALP7ex8r2 CCAGGTTTTTAAAAGTTACATTTG (SEQ ID NO: 42)

Product size: 418bp

Sequencing

NALP7ex8f2 (see SEQ ID NO: 26 above)

NALP7ex8r2 (see SEQ ID NO: 27 above)

Exon 9:

PCR

NALP7ex9-a CTTCACAGGGCGTTAGCCAGAGG (SEQ ID NO: 28)

NALP7ex9b CCAGCCCGGGAAAGATGACAAGA (SEQ ID NO: 29)

Product size: 456bp

Sequencing

NALP7ex9-a (see SEQ ID NO: 28 above)

NALP7ex9b (see SEQ ID NO: 29 above)

Exon 10:

PCR

NALP7ex10afwd AAGGTGCTGGGGCTACAGGTGTCT (SEQ ID NO: 30)

NALP7ex10arev GCCAACATGGTGAAACCCCTCTC (SEQ ID NO: 31)

Product size: 787bp

Sequencing

NALP7ex10afwd (see SEQ ID NO: 30 above)

NALP7ex10aseq_r AAACCCATACCTGAGTAT (SEQ ID NO: 40)

Exon 11:

PCR

NALP7ex11 fwd CTGTCCCCCAGAAAATCCCAAAAAC (SEQ ID NO: 32)

NALP7 ex11 rev CAACCGAATCATCCCTGAACTTC (SEQ ID NO: 33)

Product size: 588bp

Sequencing

NALP7ex11 fwd (see SEQ ID NO: 32 above)

NALP7 ex11 rev (see SEQ ID NO: 33 above)

To assess the IVS3+1 G>A mutation using the restriction enzyme *Bst*NI, the following primers were used to amplify a 204 bp fragment that was digested with the enzyme:

NALPex3-fwd CCACCATGCCTGGCTGACACTTTAT (SEQ ID NO: 10)

NALPex3b2 CAAACACCAAACTCATGACCATA (SEQ ID NO: 34)

Product size: 204bp

[00148] **Example 8:** Cytokine release in peripheral mononuclear cells from patients with mutations in *NALP7:*

[00149] The ability of peripheral blood mononuclear cells (PBMCs) harbouring homozygous *NALP7* mutations to secrete interleukin-1β (IL-1β) and TNF alpha (TNFα) in response to stimulation with bacterial lipopolysaccharide (LPS) was assessed. PBMCs were isolated from patients with NALP7 mutations (MoLb1 with IVS3+1G>A and MoGe2 with R693W) and control subjects using Ficoll gradient, stimulated with 100 ng/mL of LPS for twenty hours and the supernatants were collected for cytokine quantification using ELISA. Applicant found that the concentration of IL-1β and TNFα in the supernatant of patient' PBMCs was significantly lower than that of controls (Figures 9 and 10).

[00150] Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims. Throughout this application, various references are referred to describe more fully the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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WHAT IS CLAIMED IS:

1. A method for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female subject, the method comprising detecting an alteration in the sequence of a NALP7 gene or the sequence of its mRNA or encoded polypeptide in a tissue sample from said subject relative to the sequence of a wild-type NALP7 gene or the sequence of its mRNA or encoded polypeptide, wherein said alteration indicates that the subject suffers from or has a predisposition for the reproductive condition.

2. The method of claim 1, wherein said reproductive condition is selected from gestational trophoplastic disease, gestational trophoblastic tumor, hydatidiform mole, molar pregnancy, biparental molar pregnancy, androgenetic molar pregnancy, invasive mole, choriocarcinoma, premature ovarian failure, infertility, endometriosis, implantation failure, blighted ovum, recurrent spontaneous abortions, preeclampsia, and stillbirth.

3. The method of claim 1 or 2, wherein said alteration is associated with altered splicing of a NALP7 transcript.

4. The method of claim 3, wherein said alteration results in altered splicing of exon 3, exon 7, or both, of said NALP7 gene.

5. The method of any one of claims 1 to 4, wherein said alteration occurs at a splice donor site.

6. The method of claim 5, wherein said alteration occurs at the splice donor site at the boundary of exon 3 and intron 3, the splice donor site at the boundary of exon 7 and intron 7, or both, of the NALP7 gene.

7. The method of claim 1, wherein said alteration results is associated with a loss of a cleavage site for a restriction endonuclease in the NALP7 gene.

8. The method of claim 7, wherein the restriction endonuclease is *Bst*NI.

9. The method of claim 1, wherein the alteration is at an amino acid position within the NALP7 polypeptide selected from position 693, 399, 379, 99 and 657 of the NALP7 polypeptide.

10. The method of claim 9, wherein the alteration is selected from a substitution of the C corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide and a substitution of the G corresponding to the second position of the codon for Arg 693 of the NALP7 polypeptide.

11. The method of claim 9, wherein the alteration is selected from a substitution of Arg 693 with Trp (R693W)

12. The method of claim 9, wherein the alteration is a substitution of Cys 399 with Tyr (C399Y).

13. The method of claim 9, wherein the alteration is a substitution of Lys 379 with Asn (K379N).

14. The method of claim 9, wherein the alteration is a substitution of the codon for Glu 99 with a stop codon (E99X).

15. The method of claim 9, wherein the alteration is a substitution of Asp 657 with Val (D657V).

16. The method of claim 1, wherein the alteration is selected from:

a) a substitution of G with A at the splice donor site at the boundary of exon 3 and intron 3 (IVS3+1G>A);

b) a substitution of G with A at the splice donor site at the boundary of exon 7 and intron 7 (IVS7+1G>A);

c) a substitution of C with T corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide;

d) a substitution of G with A corresponding to the second position of the codon for Cys 84 of the NALP7 polypeptide;

e) a substitution of G with A corresponding to the second position of the codon for Cys 399 of the NALP7 polypeptide;

f) a substitution of G with C corresponding to the third position of the codon for Lys 379 of the NALP7 polypeptide;

g) a substitution of G with T corresponding to the first postion of the codon for Glu 99 of the NALP7 polypeptide; and

h) a substitution of A with T corresponding to the second postion of the codon for Asp 657 of the NALP7 polypeptide.

17. The method of claim 1, further comprising amplification of a nucleic acid sequence suspected of comprising the alteration in the sample prior to the detection of the alteration.

18. The method of claim 1, wherein detection of the alteration is performed using a method selected from:

a) sequencing of the NALP7 nucleic acid sequence;

b) hybridization of a nucleic acid probe capable of specifically hybridizing to a NALP7 nucleic acid sequence comprising the alteration and not to a corresponding wild-type NALP7 nucleic acid sequence;

c) restriction fragment length polymorphism analysis (RFLP);

d) amplified fragment length polymorphism PCR (AFLP-PCR);

e) amplification of a nucleic acid fragment comprising a NALP7 nucleic acid sequence using a primer specific for the alteration, wherein the primer produces an amplified product if the alteration is present and does not produce the same amplified product when a corresponding wild-type NALP7 nucleic acid sequence is used as a template for amplification;

f) sequencing of the NALP7 polypeptide;

g) digestion of the NALP7 polypeptide followed by mass spectrometry or HPLC analysis of the peptide fragments, wherein the alteration of the NALP7 polypeptide results in an altered mass spectrometry or HPLC spectrum as compared to wild-type NALP7 polypeptide; and

h) immunodetection using an immunological reagent which exhibits altered immunoreactivity with a NALP7 polypeptide comprising the alteration relative to a corresponding wild-type NALP7 polypeptide.

19. The method of claim 18, wherein said primer comprises a nucleotide sequence selected from SEQ ID NOs: 6-42.

20. A nucleic acid probe capable of specifically hybridizing to an altered NALP7 nucleotide sequence and not to a corresponding wild-type NALP7 nucleotide sequence.

21. A primer capable of specifically producing an amplified product from a template comprising an altered NALP7 nucleotide sequence and which does not produce the same amplified product from a template comprising a corresponding wild-type NALP7 nucleotide sequence.

22. The primer of claim 21, wherein said primer comprises a nucleotide sequence selected from SEQ ID NOs: 6-42.

23. An isolated altered NALP7 nucleic acid or fragment thereof, wherein said altered NALP7 nucleic acid or fragment thereof comprises a nucleotide sequence comprising an alteration relative to the nucleotide sequence of a wild-type NALP7 nucleic acid or fragment thereof.

24. An isolated nucleic acid comprising a sequence that encodes an altered NALP7 polypeptide or fragment thereof.

25. The isolated nucleic acid of claim 23 wherein the nucleic acid comprises an altered NALP7 nucleotide sequence comprising an alteration associated with altered splicing of a NALP7 transcript.

26. The isolated nucleic acid of claim 25, wherein the alteration results in altered splicing of exon 3, exon 7, or both, of said NALP7 gene.

27. The isolated nucleic acid of claim 26, wherein the alteration occurs at a splice donor site.

28. The isolated nucleic acid of claim 27, wherein the alteration occurs at the splice donor site at the boundary of exon 3 and intron 3, the splice donor site at the boundary of exon 7 and intron 7, or both, of the NALP7 gene.

29. The isolated nucleic acid of claim 23, wherein the alteration results is associated with a loss of a cleavage site for a restriction endonuclease in the NALP7 gene.

30. The isolated nucleic acid of claim 29, wherein the restriction endonuclease is *Bst*NI.

31. The isolated nucleic acid of claim 24, wherein the alteration results in an amino acid alteration at an amino acid position of the NALP7 polypeptide selected from position 693, 399, 379, 99, 84, and 657 of the NALP7 polypeptide.

32. The isolated nucleic acid of claim 31, wherein the alteration is selected from a substitution of the C corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide.

33. The isolated nucleic acid of claim 31, wherein the amino acid alteration is selected from a substitution of Arg 693 with Trp (R693W) .

34. The isolated nucleic acid of claim 31, wherein the alteration is a substitution of Cys 399 with Tyr (C399Y).

35. The isolated nucleic acid of claim 31, wherein the alteration is a substitution of Lys 379 with Asn (K379N).

36. The isolated nucleic acid of claim 31, wherein the alteration is a substitution of the codon for Glu 99 with a stop codon (E99X).

37. The isolated nucleic acid of claim 31, wherein the alteration is a substitution of Asp 657 with Val (D657V).

38. The isolated nucleic acid of claim 25, wherein the sequence comprises an alteration selected from:

a) a substitution of G with A at the splice donor site at the boundary of exon 3 and intron 3 (IVS3+1G>A);

b) a substitution of G with A at the splice donor site at the boundary of exon 7 and intron 7 (IVS7+1G>A);

c) a substitution of C with T corresponding to the first position of the codon for Arg 693 of the NALP7 polypeptide;

d) a substitution of G with A corresponding to the second position of the codon for Cys 84 of the NALP7 polypeptide;

e) a substitution of G with A corresponding to the second position of the codon for Cys 399 of the NALP7 polypeptide;

f) a substitution of G with C corresponding to the third position of the codon for Lys 379 of the NALP7 polypeptide;

g) a substitution of G with T corresponding to the first postion of the codon for Glu 99 of the NALP7 polypeptide; and

h) a substitution of A with T corresponding to the second postion of the codon for Asp 657 of the NALP7 polypeptide.

39. A replicative cloning vector comprising the isolated nucleic acid of claim 23 or 24 and a replicon operative in a host cell.

40. An expression vector comprising the isolated nucleic acid of claim 23 or 24 operably linked to a transcriptional regulatory element.

41. A host cell transformed with the vector of claim 39 or 40.

42. An isolated altered NALP7 polypeptide encoded by the isolated nucleic acid of claim 23 or 24.

43. An antibody capable of altered immunoreactivity with an altered NALP7 polypeptide relative to a corresponding wild-type NALP7 polypeptide.

44. A method of identifying a compound for restoring defective immune function associated with a reproductive condition, said method comprising determining whether cytokine release of an immune cell comprising an altered NALP7 nucleic acid or polypeptide is increased in the presence of a test compound relative to in the absence of said test compound; wherein said increase is indicative that said test compound may be used for restoring defective immune function associated with a reproductive condition.

45. The method of claim 44, wherein the immune cell is a lymphocyte or monocyte.

46. The method of claim 44, wherein the immune cell is a peripheral blood mononuclear cell (PBMC).

47. The method of any one of claims 44 to 46, wherein the reproductive condition is selected from gestational trophoplastic disease, gestational trophoblastic tumor, hydatidiform mole, molar pregnancy, biparental molar pregnancy, androgenetic molar pregnancy, invasive mole, choriocarcinoma, premature ovarian failure, infertility, endometriosis, implantation failure, blighted ovum, recurrent spontaneous abortions, preeclampsia, and stillbirth.

48. The method of any one of claims 44 to 47, wherein the cytokine is selected from interleukin-1β (IL-1 β) and TNF alpha (TNFα).

49. A kit for diagnosing a reproductive condition or a predisposition for a reproductive condition in a female subject, said kit comprising means for detection of an alteration in the sequence of a NALP7 gene or the sequence of its mRNA or encoded polypeptide in a tissue sample from said subject relative to the sequence of a corresponding wild-type NALP7 gene or the sequence of its mRNA or encoded polypeptide.

50. The kit of claim 48, wherein said means for detection is selected from the probe of claim 20, the primer of claim 21 and the antibody of claim 43.

51. The kit of claim 48 or 49, wherein the reproductive condition is selected from gestational trophoplastic disease, gestational trophoblastic tumor, hydatidiform mole, molar pregnancy, biparental molar pregnancy, androgenetic molar pregnancy, invasive mole, choriocarcinoma, premature ovarian failure, infertility, endometriosis, implantation failure, blighted ovum, recurrent spontaneous abortions, preeclampsia, and stillbirth.

52. The kit of any one of claims 48 to 50, further comprising means to determine cytokine release of an immune cell of said subject.

53. The kit of claim 51, wherein the immune cell is a lymphocyte or monocyte.

54. The kit of claim 51, wherein the immune cell is a peripheral blood mononuclear cell (PBMC).

55. The kit of any one of claims 51 to 53, wherein the cytokine is selected from interleukin-1β (IL-1 β) and TNF alpha (TNFα).

56. The method of any one of claims 1 to 19, further comprising determining cytokine release of an immune cell of said subject, wherein a decrease in cytokine release relative to a control level of cytokine release is further indicative that the subject suffers from or has a predisposition for the reproductive condition.

57. The method of claim 55, wherein the control level is selected from an established standard and a level of cytokine release of an immune cell comprising a wild-type NALP7 nucleic acid.

58. The method of claim 55 or 56, wherein the immune cell is a lymphocyte or monocyte.

59. The method of claim 55 or 56, wherein the immune cell is a peripheral blood mononuclear cell (PBMC).

60. The method of any one of claims 55 to 58, wherein the cytokine is selected from interleukin-1β (IL-1 β) and TNF alpha (TNFα).

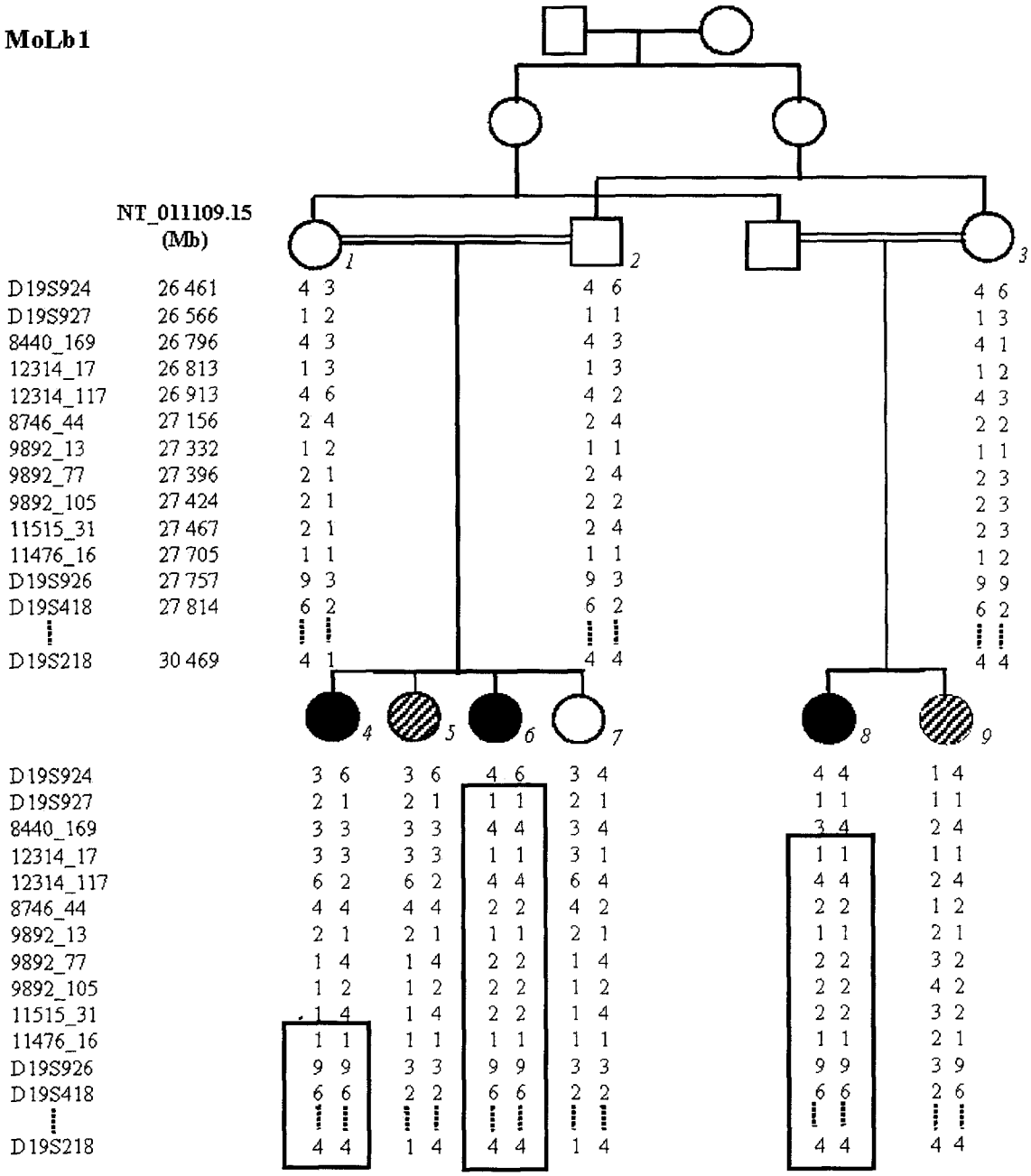

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Figure 1

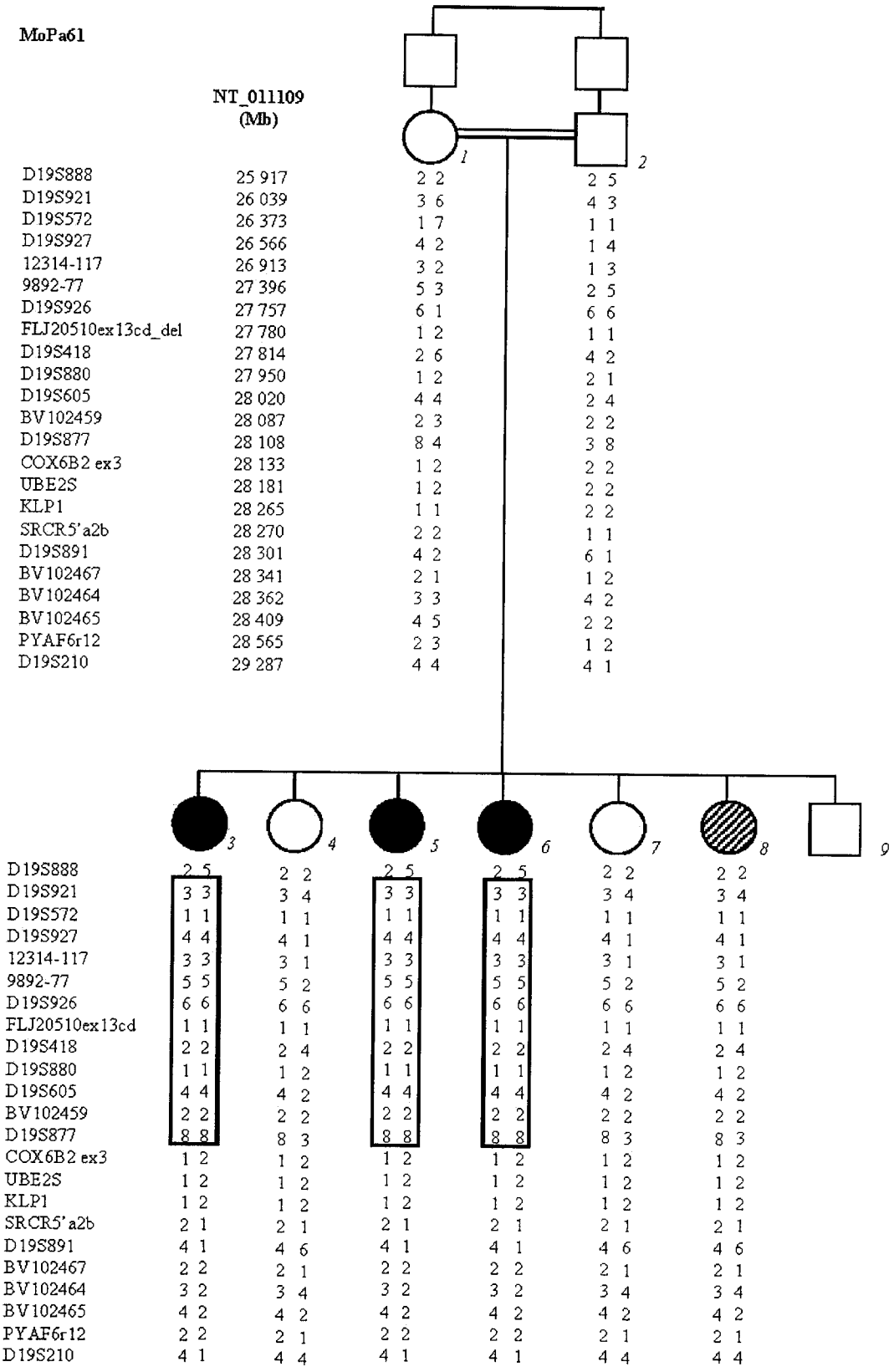

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Figure 2

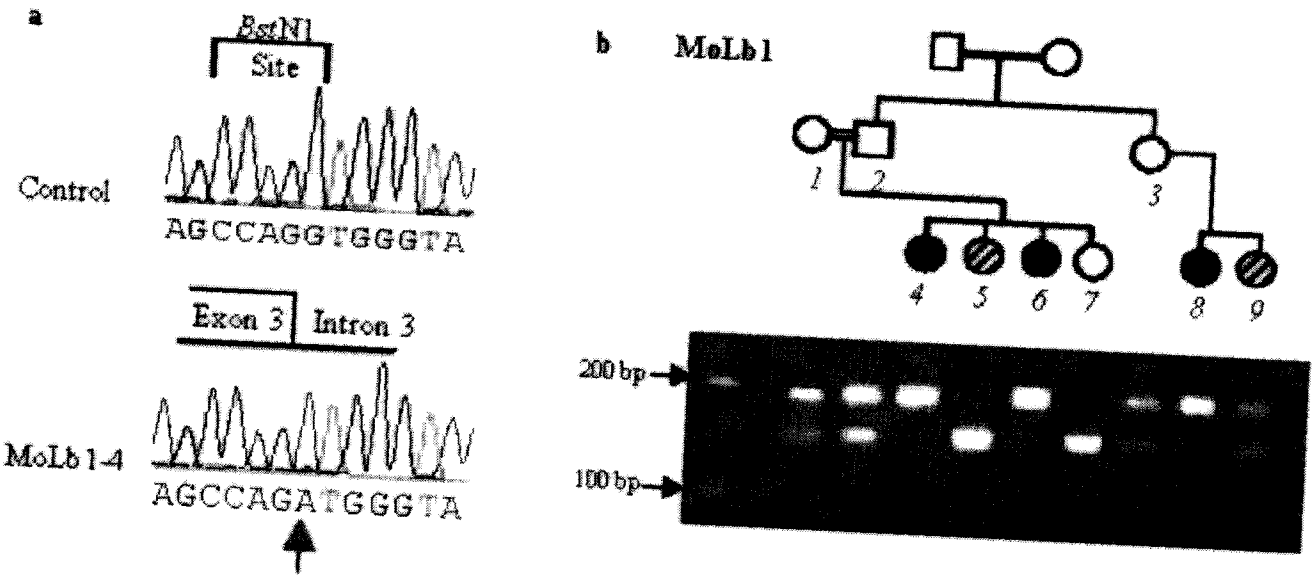

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Intron 3
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Figure 3

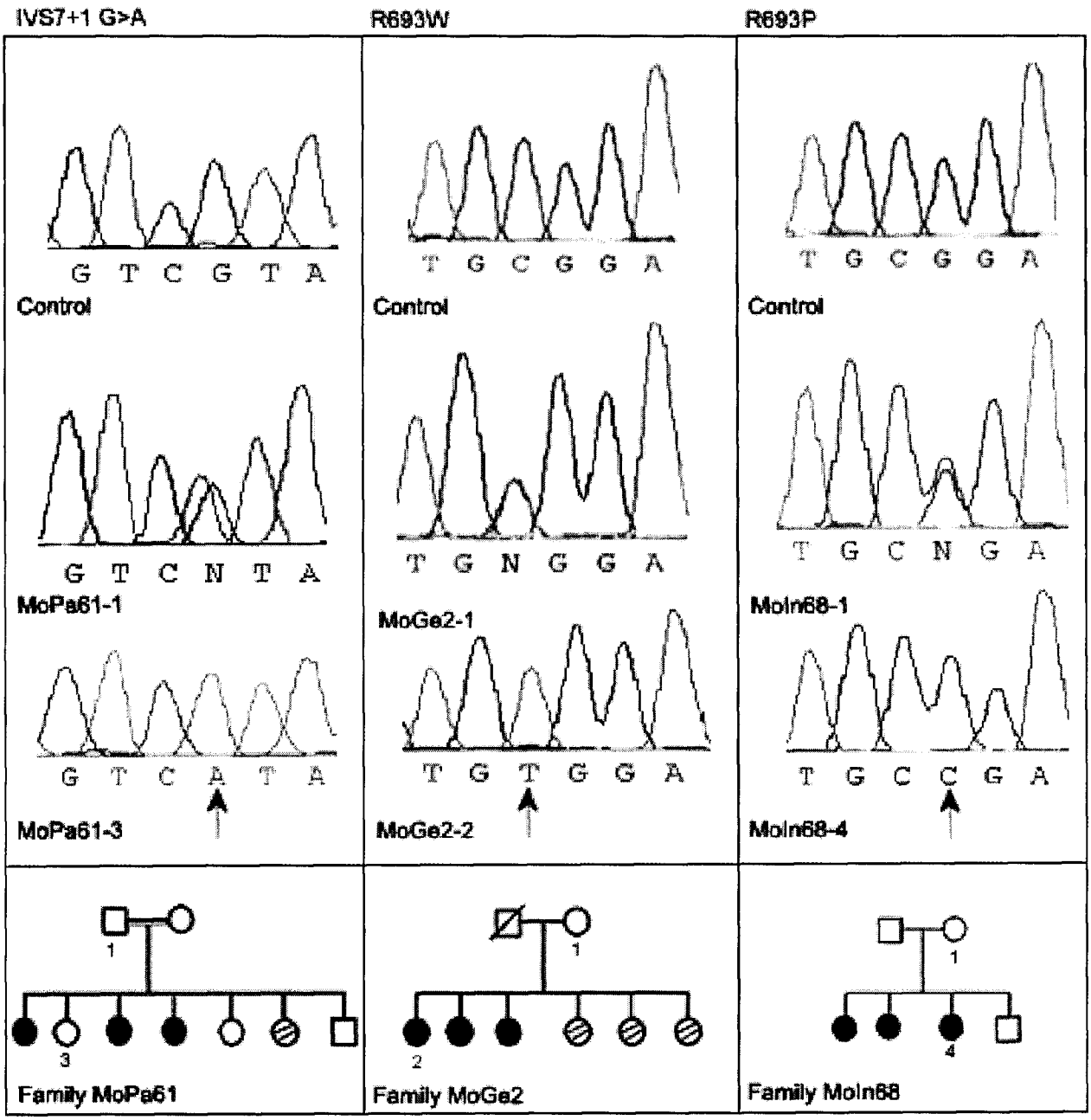

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Figure 4

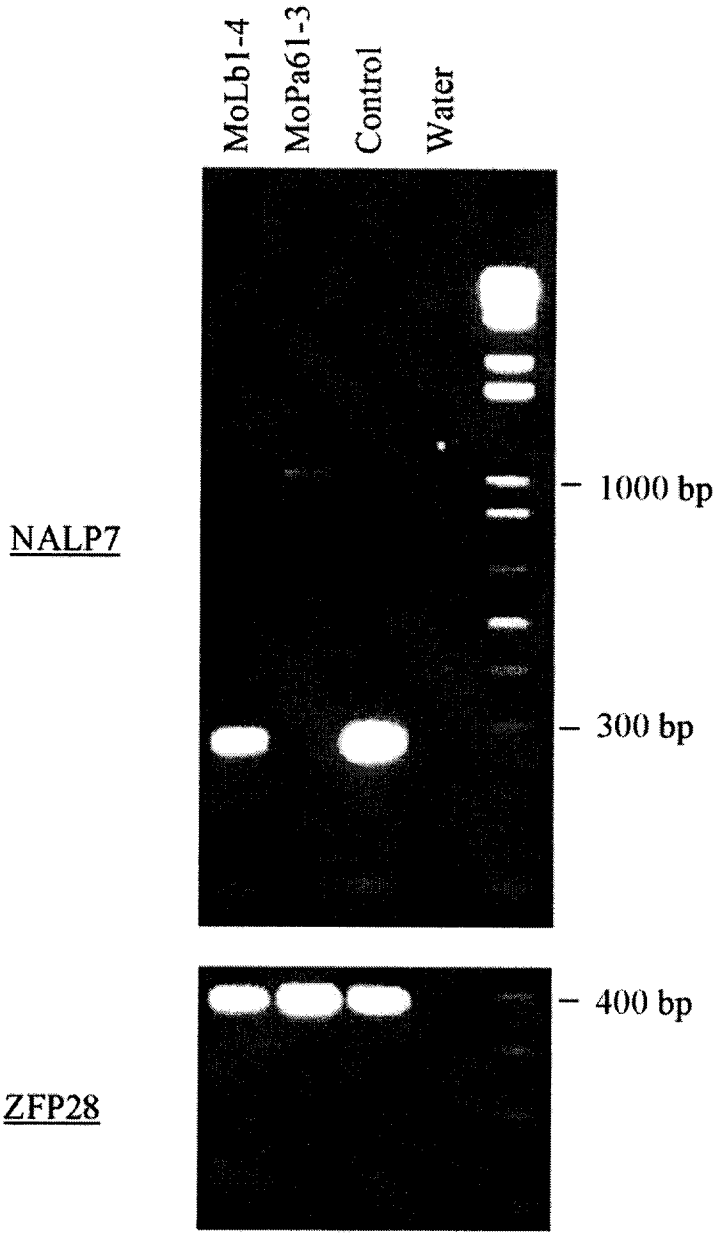

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Figure 5

NALP7 genomic DNA sequence from human chromosome 19 genomic contig. (SEQ ID NO: 1; GenBank accession No. NT_011109)

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Figure 6

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Figure 6 continued

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 8521 gaggttgcag tgagccgaga tcgcaccact gcactccagc ctgggcgaca gaacaagact
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Figure 6 : continued

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14461 tcccaccttg gcctcccaag tagctggaat tacagatgcc tgccaccatg cctggctaat
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Figure 6 : continued

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18241 ca
```

Figure 6 : continued

DNA Sequence of Human NALP7, 980 amino acid isoform (SEQ ID NO: 2; GenBank accession No. AY154462)

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 121 gaacgaggat gaattaaaga gtttcaaatc ccttttatgg gcttttcccc tcgaagacgt
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3241 tgcaa
```

Figure 7

Polypeptide sequence of Human NALP7, 980 amino acid isoform (SEQ ID NO: 3; GenBank accession No. AY154462; Swiss-Prot accession No. Q8WX94)

```
   1 mtspqlewtl qtlleqlned elksfksllw afpledvlqk tpwseveead gkklaeilvn
  61 tssenwirna tvnileemnl telckmakae mmedgqvqei dnpelgdaee dselakpgek
 121 egwrnsmekq slvwkntfwq gdidnfhddv tlrnqrfipf lnprtprklt pytvvlhgpa
 181 gvgkttlakk cmldwtdcnl sptlryafyl sckelsrmgp csfaeliskd wpelqddips
 241 ilaqaqrilf vvdgldelkv ppgaliqdic gdwekkkpvp vllgsllkrk mlpraallvt
 301 trpralrdlq llaqqpiyvr vegfleedrr ayflrhfgde dqamrafelm rsnaalfqlg
 361 sapavcwivc ttlklqmekg edpvptcltr tglflrflcs rfpqgaqlrg alrtlsllaa
 421 qglwaqmsvf hredlerlgv qesdlrlfld gdilrqdrvs kgcysfihls fqqfltalfy
 481 alekeegedr dghawdigdv qkllsgeerl knpdliqvgh flfglanekr akeleatfgc
 541 rmspdikqel lqckahlhan kplsvtdlke vlgclyesqe eelakvvvap fkeisihltn
 601 tsevmhcsfs lkhcqdlqkl slqvakgvfl enymdfeldi eferctylti pnwarqdlrs
 661 lrlwtdfcsl fssnsnlkfl evkqsflsds svrilcdhvt rstchlqkve iknvtpdtay
 721 rdfclafigk ktlthltlag hiewertmml mlcdllrnhk cnlqylrlgg hcatpeqwae
 781 ffyvlkanqs lkhlrlsanv lldegamlly ktmtrpkhfl qmlslencrl teasckdlaa
 841 vlvvskklth lclaknpigd tgvkflcegl sypdcklqtl vlqqcsitkl gcrylsealq
 901 eacsltnldl sinqiarglw ilcqalenpn cnlkhlrlkt yetnleikkl leevkeknpk
 961 ltidcnasga tappccdffc
```

Figure 7 : continued

DNA Sequence of Human NALP7, 1009 amino acid isoform (SEQ ID NO: 4; GenBank accession No. NM_139176)

```
   1 caggctggaa gcaagacctg acctgaggga gttcttcagc cttaacctaa ggtctcatac
  61 tcggagcact atgacatcgc cccagctaga gtggactctg cagacccttc tggagcagct
 121 gaacgaggat gaattaaaga gtttcaaatc ccttttatgg gcttttcccc tcgaagacgt
 181 gctacagaag accccatggt ctgaggtgga agaggctgat ggcaagaaac tggcagaaat
 241 tctggtcaac acctcctcag aaaattggat aaggaatgcg actgtgaaca tcttggaaga
 301 gatgaatctc acggaattgt gtaagatggc aaaggctgag atgatggagg acggacaggt
 361 gcaagaaata gataatcctg agctgggaga tgcagaagaa gactcggagt tagcaaagcc
 421 aggtgaaaag gaaggatgga gaaattcaat ggagaaacag tctttggtct ggaagaacac
 481 cttttggcaa ggagacattg acaatttcca tgacgacgtc actctgagaa accaacggtt
 541 cattccattc ttgaatccca gaacacccag gaagctaaca ccttacacgg tggtgctgca
 601 cggccccgca ggcgtgggga aaaccacgct ggccaaaaag tgtatgctgg actggacaga
 661 ctgcaacctc agcccgacgc tcagatacgc gttctacctc agctgcaagg agctcagccg
 721 catgggcccc tgcagttttg cagagctgat ctccaaagac tggcctgaat tgcaggatga
 781 cattccaagc atcctagccc aagcacagag aatcctgttc gtggtcgatg gccttgatga
 841 gctgaaagtc ccacctgggg cgctgatcca ggacatctgc ggggactggg agaagaagaa
 901 gccggtgccc gtcctcctgg ggagtttgct gaagaggaag atgttaccca gggcagcctt
 961 gctggtcacc acgcggccca gggcactgag ggacctccag ctcctggcgc agcagccgat
1021 ctacgtaagg gtggagggct tcctggagga ggacaggagg gcctatttcc tgagacactt
1081 tggagacgag gaccaagcca tgcgtgcctt tgagctaatg aggagcaacg cggccctgtt
1141 ccagctgggc tcggcccccg cggtgtgctg gattgtgtgc acgactctga agctgcagat
1201 ggagaagggg gaggacccgg tccccacctg cctcacccgc acggggctgt tcctgcgttt
1261 cctctgcagc cggttcccgc agggcgcaca gctgcggggc gcgctgcgga cgctgagcct
1321 cctggccgcg cagggcctgt gggcgcagat gtccgtgttc caccgagagg acctggaaag
1381 gctcggggtg caggagtccg acctccgtct gttcctggac ggagacatcc tccgccagga
1441 cagagtctcc aaaggctgct actccttcat ccacctcagc ttccagcagt ttctcactgc
1501 cctgttctac gccctggaga aggaggaggg ggaggacagg gacggccacg cctgggacat
1561 cggggacgta cagaagctgc tttccggaga agaaagactc aagaaccccg acctgattca
1621 agtaggacac ttcttattcg gcctcgctaa cgagaagaga gccaaggagt tggaggccac
1681 ttttggctgc cggatgtcac cggacatcaa acaggaattg ctgcaatgca aagcacatct
1741 tcatgcaaat aagcccttat ccgtgaccga cctgaaggag gtcttgggct gcctgtatga
1801 gtctcaggag gaggagctgg cgaaggtggt ggtggccccg ttcaaggaaa tttctattca
1861 cctgacaaat acttctgaag tgatgcattg ttccttcagc ctgaagcatt gtcaagactt
1921 gcagaaactc tcactgcagg tagcaaaggg ggtgttcctg gagaattaca tggattttga
1981 actggacatt gaatttgaaa gctcaaacag caacctcaag tttctggaag tgaaacaaag
2041 cttcctgagt gactcttctg tgcggattct ttgtgaccac gtaacccgta gcacctgtca
2101 tctgcagaaa gtggagatta aaaacgtcac ccctgacacc gcgtaccggg acttctgtct
2161 tgctttcatt gggaagaaga ccctcacgca cctgaccctg gcagggcaca tcgagtggga
2221 acgcacgatg atgctgatgc tgtgtgacct gctcagaaat cataaatgca acctgcagta
2281 cctgaggttg ggaggtcact gtgccacccc ggagcagtgg gctgaattct tctatgtcct
2341 caaagccaac cagtccctga agcacctgcg tctctcagcc aatgtgctcc tggatgaggg
2401 tgccatgttg ctgtacaaga ccatgacacg cccaaaacac ttcctgcaga tgttgtcgtt
2461 ggaaaactgt cgtcttacag aagccagttg caaggacctt gctgctgtct tggttgtcag
2521 caagaagctg acacacctgt gcttggccaa gaaccccatt ggggatacag gggtgaagtt
2581 tctgtgtgag ggcttgagtt accctgattg taaactgcag accttggtgt tacagcaatg
2641 cagcataacc aagcttggct gtagatatct ctcagaggcg ctccaagaag cctgcagcct
2701 cacaaacctg gacttgagta tcaaccagat agctcgtgga ttgtggattc tctgtcaggc
2761 attagagaat ccaaactgta acctaaaaca cctacgcctc tggagctgct ccctcatgcc
2821 tttctattgt cagcatcttg gatctgctct cctcagcaat cagaagcttg aaactctgga
2881 cctgggccag aatcatttgt ggaagagtgg cataattaag ctctttgggg ttctaagaca
2941 aagaactgga tccttgaaga tactcaggtt gaagacctat gaaactaatt tggaaatcaa
3001 gaagctgttg gaggaagtga aagaaaagaa tcccaagctg actattgatt gcaatgcttc
3061 cggggcaacg gcacctccgt gctgtgactt tttttgctga gcagcctggg atcgctctac
3121 gaattacaca ggaagcggga ttcgggtctc taagatgtct tatgaatgca ggtcagaggg
3181 tcacatgtta acactagagt ctgtcgagag gtaggatttg acactggttt tctcactatt
3241 tttgggagat tctgcacgag tcacgcaccc ccttcacatg acgctatgta ctttctcaca
3301 gggataataa agttagagca ctctcgttgc a
```

Figure 8

Polypeptide of Human NALP7, 1009 amino acid isoform (SEQ ID NO: 5; GenBank accession No. NM_139176, DAA01246.1)

```
   1 mtspqlewtl qtlleqlned elksfksllw afpledvlqk tpwseveead geklaeilvn
  61 tssenwirna tvnileemnl telckmakae mmedgqvqei dnpelgdaee dselakpgek
 121 egwrnsmekq slvwkntfwq gdidnfhddv tlrnqrfipf lnprtprklt pytvvlhgpa
 181 gvgkttlakk cmldwtdcnl sptlryafyl sckelsrmgp csfaeliskd wpelqddips
 241 ilaqaqrilf vvdgldelkv ppgaliqdic gdwekkkpvp vllgsllkrk mlpraallvt
 301 trpralrdlq llaqqpiyvr vegfleedrr ayflrhfgde dqamrafelm rsnaalfqlg
 361 sapavcwivc ttlklqmekg edpvptcltr tglflrflcs rfpqgaqlrg alrtlsllaa
 421 qglwaqmsvf hredlerlgv qesdlrlfld gdilrqdrvs kgcysfihls fqqfltalfy
 481 alekeegedr dghawdigdv qkllsgeerl knpdliqvgh flfglanekr akeleatfgc
 541 rmspdikqel lqckahlhan kplsvtdlke vlgclyesqe eelakvvvap fkeisihltn
 601 tsevmhcsfs lkhcqdlqkl slqvakgvfl enymdfeldi efessnsnlk flevkqsfls
 661 dssvrilcdh vtrstchlqk veiknvtpdt ayrdfclafi gkktlthltl aghiewertm
 721 mlmlcdllrn hkcnlqylrl gghcatpeqw aeffyvlkan qslkhlrlsa nvlldegaml
 781 lyktmtrpkh flqmlslenc rlteasckdl aavlvvskkl thlclaknpi gdtgvkflce
 841 glsypdcklq tlvlqqcsit klgcrylsea lqeacsltnl dlsinqiarg lwilcqalen
 901 pncnlkhlrl wscslmpfyc qhlgsallsn qkletldlgq nhlwksgiik lfgvlrqrtg
 961 slkilrlkty etnleikkll eevkeknpkl tidcnasgat appccdffc
```

Figure 8 : continued

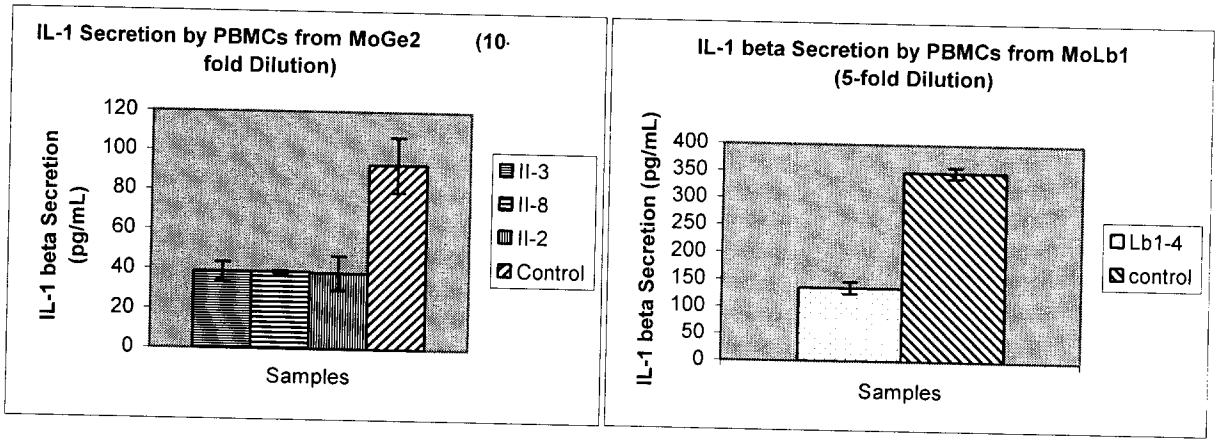

IL-1 Secretion by PBMCs from MoGe2 (10-fold Dilution)
IL-1 beta Secretion (pg/mL)
120
100
80
60
40
20
0
Samples
Il-3
Il-8
Il-2
Control
IL-1 beta Secretion by PBMCs from MoLb1 (5-fold Dilution)
IL-1 beta Secretion (pg/mL)
400
350
300
250
200
150
100
50
0
Samples
Lb1-4
control

Figure 9

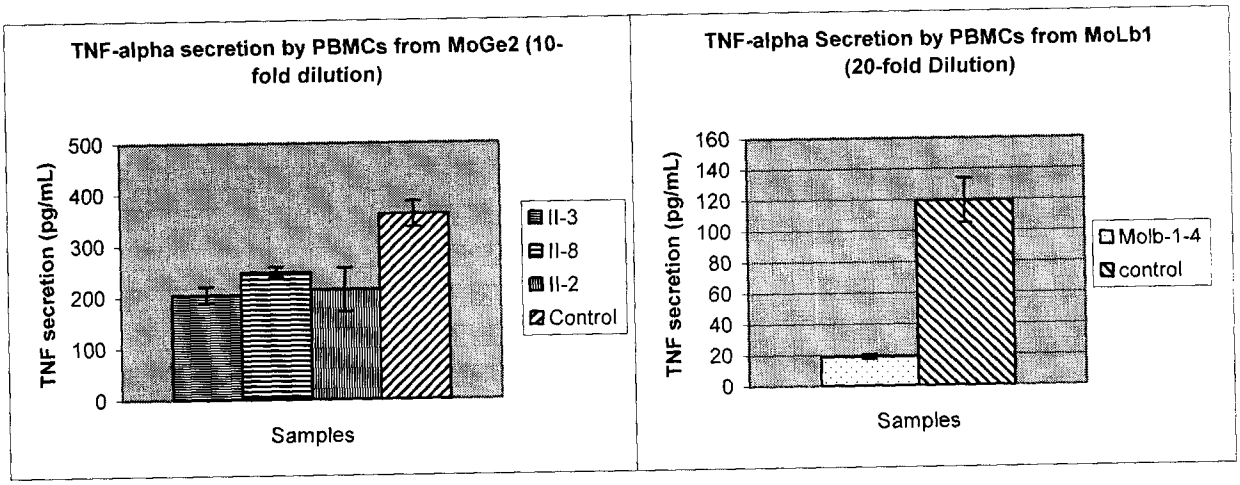

TNF-alpha secretion by PBMCs from MoGe2 (10-fold dilution)
TNF secretion (pg/mL)
500
400
300
200
100
0
Samples
Il-3
Il-8
Il-2
Control
TNF-alpha Secretion by PBMCs from MoLb1 (20-fold Dilution)
TNF secretion (pg/mL)
160
140
120
100
80
60
40
20
0
Samples
Molb-1-4
control

Figure 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001256

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12N 15/12*** (2006.01), ***C07K 16/18*** (2006.01), ***C07K 14/435*** (2006.01), ***C12N 1/21*** (2006.01), ***C12N 15/63*** (2006.01), ***C12N 5/10*** (2006.01), ***C12P 19/34*** (2006.01), ***C12Q 1/48*** (2006.01), ***C12Q 1/68*** (2006.01), ***G01N 33/53*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: ***C12N 15/12*** (2006.01), ***C07K 16/18*** (2006.01), ***C07K 14/435*** (2006.01), ***C12N 1/21*** (2006.01), ***C12N 15/63*** (2006.01), ***C12N 5/10*** (2006.01), ***C12P 19/34*** (2006.01), ***C12Q 1/48*** (2006.01), ***C12Q 1/68*** (2006.01), ***G01N 33/53*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Medline, GenomeQuest, STN (CAplus, BIOSIS), Delphion, Canadian Patent Database, USPTO, Espacenet
Search terms: NALP7, PYPAF3, NACHT, NOD, reprod?, hydatidiform mole, splice, variant, mutation, gestational trophoplastic disease, gestational trophoblastic tumour, molar pregnancy, invasive mole, choriocarcinoma, blighted ovum, recurrent spontaneous abortion, stillbirth

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	MURDOCH, S. et al. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nature Genetics. March 2006, Vol. 38, No. 3, pages 300-302, ISSN 1061-4036. whole document	1-11, 16-18, 26-28, 30-33, 38
A	MOGLABEY, Y.B. et al. Genetic mapping of a maternal locus responsible for familial hydatidiform moles. Human Molecular Genetics. April 1999, Vol. 8, No. 4, pages 667-671, ISSN 0964-6906. whole document cited in application	1-19, 22, 26-28, 30-38, 43-60
A	HODGES, M.D. et al. Genetic refinement and physical mapping of a biparental complete hydatidiform mole locus on chromosome 19q13.4. Journal of Medical Genetics. August 2003, Vol. 40, No. 8, e95, pages 1-6, ISSN 0022-2593. whole document cited in application	1-19, 22, 26-28, 30-38, 43

[ ] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 October 2006 (02.10.2006)	15 November 2006 (15-11-2006)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Neena Kushwaha (819) 934-7928

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001256

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [ ] Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :

2. [X] Claim Nos. : 20, 21, 23-25, 29, 39-42
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

The probe of claim 20, the primer of claim 21, the isolated nucleic acid of claims 23-25 and 29, vectors and a host cell comprising said isolated nucleic acid (claims 39-41), and the polypeptide of claim 42, encompass products with insufficient technical features. For example, based on the definition of the term "alteration" found on page 14 of the **(CONTINUED ON EXTRA SHEET)**

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.