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[54] **CANCER SUSCEPTIBILITY MUTATIONS OF BRCA2**

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[52] **U.S. Cl.** **435/6**; 536/24.31; 536/24.33

[58] **Field of Search** 536/24.33, 24.31, 536/24.5; 435/6, 91.2, 320.1

[56] **References Cited**

U.S. PATENT DOCUMENTS

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[57] **ABSTRACT**

New mutations have been found in the BRCA2 gene. The mutations are located at nucleotide numbers 2192, 3772, 5193, 5374, 6495 or 6909 of the published nucleotide sequence of BRCA2 gene. A process for identifying a sequence variation in a BRCA2 polynucleotide sequence is disclosed. The identification process includes allele specific sequence-based assays of known sequence variations. The methods can be used for efficient, and accurate detection of a mutation in a test BRCA2 gene sample.

44 Claims, No Drawings

CANCER SUSCEPTIBILITY MUTATIONS OF BRCA2

This application is in part based on provisional patent application 60/059,595 filed Sep. 23, 1997, the contents are incorporated by reference.

FIELD OF THE INVENTION

This invention relates to the breast cancer succeptibility gene BRCA2. More specifically, this invention detects germline mutations of the BRCA2 gene that are associated with a predisposition to breast, ovarian and associated cancers. Methods and reagents for detecting the presence of these mutations are included.

BACKGROUND OF THE INVENTION

BRCA2, located on chromosome 13q12-q13, consists of over 70 kb of genomic DNA. The coding sequence produces a protein of 3,418 amino acids. Although most of the exons are small, exons 10 and 11 represent approximately 60% of the entire coding region. BRCA2 is thought to be a tumor suppressor gene associated with breast and ovarian cancer. Thus mutantions which form an altered tumor suppressor or altered concentrations of tumor suppressor may be indicative of a higher succeptibility to certain cancers.

The nucleotide sequence for at least one BRCA2 gene is known and is reported in GENBANK accession Number U43746. The BRCA2 gene sequence is available on the Breast Cancer Information Core.

Germline mutations of BRCA2 are predicted to account for approximately 35% of families with multiple case, early onset female breast cancer, and they are also associated with an increased risk of male breast cancer, ovarian cancer, prostrate cancer and pancreatic cancer.

The location of one or more mutations of the BRCA2 gene provides a promising approach to reducing the high incidence and mortality associated with breast and ovarian cancer through the early detection of women at high risk. These women, once identified, can be targeted for more aggressive prevention programs. Screening is carried out by a variety of methods which include karyotyping, probe binding and DNA sequencing. In such cases where one or only a few known mutations are responsible for the disease, such as testing family members, methods for detecting the mutations are targeted to the site within the gene at which they are known to occur.

Many mutations and normal polymorphisms have already been reported in the BRCA2 gene. A world wide web site has been built to facilitate the detection and characterization of alterations in breast cancer susceptibility genes. Such mutations in BRCA2 can be accessed through the Breast Cancer Information Core at:
HTTP://www.nchgr.nih.gov/dir/lab_transfer/bic.

While mutations occur throughtout the BRCA2 gene, there is a need for a high sample number (throughput), sensitivity, accuracy and cost effectiveness. Identification of mutations of the BRCA2 gene would allow more widespread diagnostic screening for hereditary breast and ovarian cancer than is currently possible and permit identification of functional areas deduced from the mutational spectrum observed.

SUMMARY OF THE INVENTION

The present invention is based on the discovery of six mutations in the BRCA2 gene sequence which is associated with susceptibility to and development of breast and ovarian cancer. Specifically, mutations located at nucleotide numbers 2192, 3772, 5193, 5374, 6495 and 6909 have been discovered.

It is an object of the invention to provide a method for determining a predisposition or higher susceptibility to breast, ovarian and other cancers.

It is another object of the invention to provide primers for detecting and amplifying a region of DNA which contains the BRAC2 mutations.

It is another object of the invention to provide probes for detecting a region of DNA which contains the BRAC2 mutations.

It is a further object of the invention to provide a method of characterizing and classifying a tumor and determining a therapy dependant upon the type of mutation(s) present.

It is also an object of the present invention to provide a mutant BRCA2 gene and expressed mutant protein for drug development, gene therapy and other uses to prevent or amelorate the effects of or resulting from the mutant BRCA2 gene.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

For defining the present invention, the following nomenclature is used to describe the mutation due to an inconsistency in the published literature. Beaudet et al, *Human Mutations,* 2: 245–248 (1993), Antonarakis et al, *Human Mutations,* 4: 166 (1994), Cotton, *Human Mutations,* 8: 197–202 (1996), and Beutler et al, *Human Mutations,* 8: 203–206 (1996). In defining the mutation, the number indicates the nucleotide number corresponding to the BRCA2 gene sequence where the mutation first occurs. Other BRCA2 sequences (haplotypes) which are polymorphisms or genetic variations of BRCA2 may used, in which a corresponding mutation at the corresponding nucleotide number are present. Different sequence variations in a normal BRCA1 gene have been discovered previously by the inventors (U.S. Pat. No. 5,654,155) and sequence variations in a normal BRCA2 gene sequence are expected. Also note Shattuck-Eidens, et al, *Journal of the American Medical Association,* 278: p. 1242 (1997). Generally, the sense strand is referred to. For simplified identification purposes of this application, reference is to the BRCA2 sequence referenced above, however the invention is equally applicable to all of the normal BRCA2 sequences.

Insertion mutations are indicated by "ins" and deletion mutations are indicated by "del". The letters after "ins" or "del" refer to the nucleotide(s) which were inserted or deleted. Insertions and deletions above two nucleotides are indicated by the number of nucleotides inserted or deleted. When the mutation results in one nucleotide being substituted for another, the nucleotide of the BRCA2 gene sequence is placed to the left of the number and the nucleotide found in the mutation is placed to the right of the number.

The first mutation is referred to as C2192G. This mutation or genetic alteration causes a change in nucleotide number 2192 from C to G resulting in codon 655 being changed from proline to arginine. Any amino acid change can have a dramatic change in biological activity. Some people believe that since proline can form a turn in the chain of amino acids in the protein, the removal of this turn, particularly when substituted with a charged amino acid may change the three dimentional configuration of the protein or at least may negatively affect on the biological activity of the resulting protein.

The second mutation is referred to as 3772delTT. This mutation deletes TT at nucleotide number 3772 causing a frameshift mutation and forming an in-frame stop codon at codon 1182. It has been demonstrated that a truncated, and most likely non-functional, protein has been produced by this mutation.

The third mutation is referred to as C5193G. This mutation substitutes G for C at nucleotide number 5193 causing a stop codon (TAG) to be formed at codon 1655. It has been demonstrated that a truncated, and most likely non-functional, protein has been produced by this mutation.

The fourth mutation is referred to as 5374del4. This mutation deletes TATG at nucleotide number 5374 causing a frameshift mutation and forming an in-frame stop at codon 1723. It has been demonstrated that a truncated, and most likely non-functional, protein has been produced by this mutation.

The fifth mutation is referred to as 6495delGC. This mutation deletes GC at nucleotide number 6495 causing a frameshift mutation and forming an in-frame stop codon at codon 2090. It has been demonstrated that a truncated, and most likely non-functional, protein has been produced by this mutation.

The sixth mutation is referred to as 6909insG. This mutation inserts a G at nucleotide number 6909 causing a frameshift mutation and forming an in-frame stop codon at codon 2232. It has been demonstrated that a truncated, and most likely non-functional, protein has been produced by this mutation.

The presence of truncated proteins was demonstrated by expression of overlapping portions of the mutant genes and measuring molecular weight by gel electrophoresis.

Useful DNA molecules according to the present invention are those which will specifically hybridize to BRCA2 sequences in the region of the C2192G, 3772delTT, C5193G, 5374del4, 6495delGC or 6909insG mutations. Typically these DNA molecules are 17 to 20 nucleotides in length (longer for large insertions) and have the nucleotide sequence corresponding to the region of the mutations at their respective nucleotide locations on the BRCA2 gene sequence. Such molecules can be labeled, according to any technique known in the art, such as with radiolabels, fluorescent labels, enzymatic labels, sequence tags, biotin, other ligands, etc.

According to another aspect of the invention, the DNA molecules, or oligonucleotides, contain one or more of the specific mutations. Generally it is preferred for each DNA probe to encompass only one mutation. Such molecules may be labeled and can be used as allele-specific oligonucleotide probes to detect the mutation of interest.

Polynucleotide containing biological samples, such as blood, can be tested to determine whether the BRCA2 gene contains one of the specific mutations listed above. To amplify the BRCA2 gene, one may use polymerase chain reaction (PCR) using primers which hybridize to the ends of the exons or to the introns flanking the exons. In the situation of exon 11, the exon is so large that using plural pairs of primers to amplify overlapping regions is preferred. Such was actually used in the Examples below.

Amplification may also be performed by a number of other techniques such as by cloning the gene and linking the BRCA2 gene or fragments thereof in the sample to a vector. "Shot gun" cloning is particularly preferred. For the purposes of this application, a vector may be any polynucleotide containing system which induces replication such as a plasmid, cosmid, virus, transposon, or portions thereof.

In one embodiment of the invention a pair of isolated oligonucleotide primers are provided.

BRCA2-11F 5'TGG TAC TTT AAT TTT GTC ACT T3' SEQ ID NO:1

BRCA2-11R 5'TGC AGG CAT GAC AGA GAA T3' SEQ ID NO:2

The designation BRCA2-11 refers to a sequence in or near exon 11 of the BRCA2 gene. F and R refer to forward and reverse.

The oligonucleotide primers are useful in directing amplification of a target polynucleotide prior to sequencing. These unique BRCA2 exon 11 oligonucleotide primers were used to scan the BRCA2 gene to find the mutations. From the sequence information, the probes were designed and produced to assay for the mutation based upon identification of the C2192G mutation.

In another embodiment of the invention a pair of isolated allele specific oligonucleotide probes are provided.

5'TGA AGA ACC AAC TTT GT3' SEQ ID NO:3

5'TGA AGA ACG AAC TTT GT3' SEQ ID NO:4

These allele specific oligonucleotides are useful in diagnosis of a subject at risk of having breast or ovarian cancer. The allele specific oligonucleotides hybridize with a target polynucleotide sequence containing the C2192G mutation. 5'TGA AGA ACC AAC TTT GT3', SEQ ID NO:3, hybridizes preferentially to the wildtype sequence and is useful as a control sequence. 5'TGA AGA ACG AAC TTT GT3', SEQ ID NO:4, is designed to hybridize preferentially to the mutant sequence.

In a second embodiment of the invention a pair of isolated oligonucleotide primers are provided.

BRCA2-11F 5'CTC AGA TGT TAT TTT CCA AGC3' SEQ ID NO:5

BRCA2-11R 5'CTG TTA AAT AAC CAG AAG CAC3' SEQ ID NO:6

The oligonucleotide primers are useful in directing amplification of a target polynucleotide prior to sequencing. These unique BRCA2 exon 11 oligonucleotide primers were used to scan the BRCA2 gene to find the mutations. From the sequence information, the probes were designed and produced to assay for the mutation based upon identification of the 3772delTT mutation.

In another embodiment of the invention a pair of isolated allele specific oligonucleotides are provided.

5'GCA AGC AAT TTG AAG GT3' SEQ ID NO:7

5'GCA AGC AAT GAA GGT AC3' SEQ ID NO:8

These allele specific oligonucleotides are useful in diagnosis of a subject at risk of having breast or ovarian cancer. The allele specific oligonucleotides hybridize with a target polynucleotide sequence containing the 3772delTT mutation. 5'GCA AGC AAT TTG AAG GT3', SEQ ID NO:7, hybridizes preferentially to the wildtype sequence and is useful as a control sequence. 5'GCA AGC AAT GAA GGT AC3', SEQ ID NO:8, is designed to hybridize preferentially to the mutant sequence.

In a third embodiment of the invention a pair of isolated oligonucleotide primers are provided.

BRCA2-11F 5'GCA AAG ACC CTA AAG TAC AG3', SEQ ID NO:9

BRCA2-11R 5'CAT CAA ATA TTC CTT CTC TAA G3', SEQ ID NO:10

The oligonucleotide primers are useful in directing amplification of a target polynucleotide prior to sequencing. These unique BRCA2 exon 11 oligonucleotide primers were used to scan the BRCA2 gene to find the mutations. From the sequence information, the probes were designed and produced to assay for the mutation based upon identification of the C5193G mutation.

In another embodiment of the invention a pair of isolated allele specific oligonucleotides are provided.

5'ACT TGT TAC ACA AAT CA3', SEQ ID NO:11

5'ACT TGT TAG ACA AAT CA3', SEQ ID NO:12

These allele specific oligonucleotides are useful in diagnosis of a subject at risk of having breast or ovarian cancer. The allele specific oligonucleotides hybridize with a target polynucleotide sequence containing the C5193G mutation. 5'ACT TGT TAC ACA AAT CA3', SEQ ID NO:11, hybridizes preferentially to the wildtype sequence and is useful as

a control sequence. 5'ACT TGT TAG ACA AAT CA3', SEQ ID NO:12, is designed to hybridize preferentially to the mutant sequence.

In a fourth embodiment of the invention a pair of isolated oligonucleotide primers are provided.

BRCA2-11F 5'GAA AAT TCA GCC TTA GC3' SEQ ID NO:13

BRCA2-11R 5'ATC AGA ATG GTA GGA AT3' SEQ ID NO:14

The oligonucleotide primers are useful in directing amplification of a target polynucleotide prior to sequencing. These unique BRCA2 exon 11 oligonucleotide primers were used to scan the BRCA2 gene to find the mutations. From the sequence information, the probes were designed and produced to assay for the mutation based upon identification of the 5374del4 mutation.

In another embodiment of the invention a pair of isolated allele specific oligonucleotides are provided.

5'ATT ATT TGT ATG AAA AT3' SEQ ID NO:15

5'ATT ATT TGA AAA TAA TT3' SEQ ID NO:16

These allele specific oligonucleotides are useful in diagnosis of a subject at risk of having breast or ovarian cancer. The allele specific oligonucleotides hybridize with a target polynucleotide sequence containing the 5374del4 mutation. 5'ATT ATT TGT ATG AAA AT3', SEQ ID NO:15, hybridizes preferentially to the wildtype sequence and is useful as a control sequence. 5'ATT ATT TGA AAA TAA TT3', SEQ ID NO:16, is designed to hybridize preferentially to the mutant sequence.

In a fifth embodiment of the invention a pair of isolated oligonucleotide primers are provided.

BRCA2-11F 5'TAC AGC AAG TGG AAA GC3' SEQ ID NO:17

BRCA2-11R 5'AAG TTT CAG TTT TAC CAA T3' SEQ ID NO:18

The oligonucleotide primers are useful in directing amplification of a target polynucleotide prior to sequencing. These unique BRCA2 exon 11 oligonucleotide primers were used to scan the BRCA2 gene to find the mutations. From the sequence information, the probes were designed and produced to assay for the mutation based upon identification of the 6495delGC mutation.

In another embodiment of the invention a pair of isolated allele specific oligonucleotides are provided.

5'GAA CTG AGC ATA GTC TT3' SEQ ID NO:19

5'GAA CTG AAT AGT CTT CA3' SEQ ID NO:20

These allele specific oligonucleotides are useful in diagnosis of a subject at risk of having breast or ovarian cancer. The allele specific oligonucleotides hybridize with a target polynucleotide sequence containing the 6495delGC mutation. 5'GAA CTG AGC ATA GTC TT3', SEQ ID NO:19, hybridizes preferentially to the wildtype sequence and is useful as a control sequence. 5'GAA CTG AAT AGT CTT CA3', SEQ ID NO:20, is designed to hybridize preferentially to the mutant sequence.

In a sixth embodiment of the invention a pair of isolated oligonucleotide primers are provided.

BRCA2-11F 5'ACT TTT TCT GAT GTT CCT GTG3' SEQ ID NO:21

BRCA2-11R 5'TAA AAA TAG TGA TTG GCA ACA3' SEQ ID NO:22

The oligonucleotide primers are useful in directing amplification of a target polynucleotide prior to sequencing. These unique BRCA2 exon 11 oligonucleotide primers were used to scan the BRCA2 gene to find the mutations. From the sequence information, the probes were designed and produced to assay for the mutation based upon identification of the 6909insG mutation.

In another embodiment of the invention a pair of isolated allele specific oligonucleotides are provided.

5'CAG AAG CAG TAG AAA TT3' SEQ ID NO:23

5'CAG AAG CAG GTA GAA AT3' SEQ ID NO:24

These allele specific oligonucleotides are useful in diagnosis of a subject at risk of having breast or ovarian cancer. The allele specific oligonucleotides hybridize with a target polynucleotide sequence containing the 6909insG mutation. 5'CAG AAG CAG TAG AAA TT3', SEQ ID NO:23, hybridizes preferentially to the wildtype sequence and is useful as a control sequence. 5'CAG AAG CAG GTA GAA AT3', SEQ ID NO:24, is designed to hybridize preferentially to the mutant sequence.

The primers of the invention embrace oligonucleotides of sufficient length and appropriate sequence to provide initiation of polymerization on a significant number of nucleic acids in the polymorphic locus.

Preferred sequences for the present invention are SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:22. Environmental conditions conducive to synthesis of extension products include the presence of nucleoside triphosphates, an agent for polymerization, such as DNA polymerase, and suitable conditions such as temperature, ionic strength and pH. The primer is preferably single stranded for maximum efficiency in amplification, but may be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent for polymerization. The exact length of primer will depend on many factors, including temperature, buffer, and nucleotide composition. The oligonucleotide primer typically contains 12–20 or more nucleotides, although it may contain fewer nucleotides.

Primers of the invention are designed to be "substantially" complementary to each strand of the genomic locus to be amplified. This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions which allow the agent for polymerization to perform. In other words, the primers should have sufficient complementarity with the 5' and 3' sequences flanking the mutation to hybridize therewith and permit amplification of the genomic locus.

Oligonucleotide primers of the invention are employed in the amplification process which is an enzymatic chain reaction that produces exponential quantities of polymorphic locus relative to the number of reaction steps involved. Typically, one primer is complementary to the negative (−) strand of the polymorphic locus and the other is complementary to the positive (+) strand. Annealing the primers to denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA polymerase I (Klenow) and nucleotides, results in newly synthesized + and − strands containing the target polymorphic locus sequence. Because these newly synthesized sequences are also templates, repeated cycles of denaturing, primer annealing, and extension results in exponential production of the region (i.e., the target polymorphic locus sequence) defined by the primers. The product of the chain reaction is a discreet nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

The oligonucleotide primers of the invention may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments thereof. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage, et al., *Tetrahedron Letters,* 22:1859–1862, (1981). One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Pat. No. 4,458,066.

Any nucleic acid specimen, in purified or nonpurified form, can be utilized as the starting nucleic acid or acids,

providing it contains, or is suspected of containing, the specific nucleic acid sequence containing the polymorphic locus. Thus, the process may amplify, for example, DNA or RNA, including messenger RNA, wherein DNA or RNA may be single stranded or double stranded. In the event that RNA is to be used as a template, enzymes, and/or conditions optimal for reverse transcribing the template to DNA would be utilized. In addition, a DNA-RNA hybrid which contains one strand of each may be utilized. A mixture of nucleic acids may also be employed, or the nucleic acids produced in a previous amplification reaction herein, using the same or different primers may be so utilized. The specific nucleic acid sequence to be amplified, i.e., the polymorphic locus, may be a fraction of a larger molecule or can be present initially as a discrete molecule, so that the specific sequence constitutes the entire nucleic acid. It is not necessary that the sequence to be amplified be present initially in a pure form; it may be a minor fraction of a complex mixture, such as contained in whole human DNA.

DNA utilized herein may be extracted from a body sample, such as blood, tissue material and the like by a variety of techniques such as that described by Maniatis, et. al. in *Molecular Cloning:A Laboratory Manual*, Cold Spring Harbor, N.Y., p 280–281, 1982). If the extracted sample is impure, it may be treated before amplification with an amount of a reagent effective to open the cells, or animal cell membranes of the sample, and to expose and/or separate the strand(s) of the nucleic acid(s). This lysing and nucleic acid denaturing step to expose and separate the strands will allow amplification to occur much more readily.

The deoxyribonucleotide triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90°–100° C. from about 1 to 10 minutes, preferably from 1 to 4 minutes. This is sufficient to denature any double strands. After this heating period, the solution is allowed to cool at a rate which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (called herein "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art. The agent for polymerization may also be added together with the other reagents if it is heat stable. This synthesis (or amplification) reaction may occur at room temperature up to a temperature above which the agent for polymerization no longer functions. Thus, for example, if DNA polymerase is used as the agent, the temperature is generally no greater than about 40° C. Thermostable DNA polymerases, such as Taq polymerase may function at a higher temperature.

The agent for polymerization may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase, polymerase muteins, reverse transcriptase, other enzymes, including heat-stable enzymes (i.e., those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation), such as Taq polymerase. The suitable enzyme will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each polymorphic locus nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths.

The newly synthesized strand and its complementary nucleic acid strand will form a double-stranded molecule under hybridizing conditions described above and this hybrid is used in subsequent steps of the process. In the next step, the newly synthesized double-stranded molecule is subjected to denaturing conditions using any of the procedures described above to provide single-stranded molecules.

The steps of denaturing, annealing, and extension product synthesis can be repeated as often as needed to amplify the target polymorphic locus nucleic acid sequence to the extent necessary for detection. The amount of the specific nucleic acid sequence produced will accumulate in an exponential fashion. PCR. A Practical Approach, ILR Press, Eds. M. J. McPherson, P. Quirke, and G. R. Taylor, 1992.

The amplification products may be detected by analyzing it by Southern blots without using radioactive probes. In such a process, for example, a small sample of DNA containing a very low level of the nucleic acid sequence of the polymorphic locus is amplified, and analyzed via a Southern blotting technique or similarly, using dot blot analysis. The use of non-radioactive probes or labels is facilitated by the high level of the amplified signal. Alternatively, probes used to detect the amplified products can be directly or indirectly detectably labeled, for example, with a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator or an enzyme. Those of ordinary skill in the art will know of other suitable labels for binding to the probe, or will be able to ascertain such, using routine experimentation. In the preferred embodiment, the amplification products are determinable by separating the mixture on an agarose gel containing ethidium bromide which causes DNA to be fluorescent.

Sequences amplified by the methods of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR, oligomer restriction (Saiki, et.al., *Bio/Technology,* 3:1008–1012, 1985), allele-specific oligonucleotide (ASO) probe analysis (Conner, et. al., *Proc. Natl. Acad. Sci. U.S.A.,* 80:278, 1983), oligonucleotide ligation assays (OLAs) (Landgren, et. al., *Science,* 241:1007, 1988), and the like. Molecular techniques for DNA analysis have been reviewed (Landgren, et. al., *Science,* 242:229–237, 1988).

Preferably, the method of amplifying is by PCR, as described herein and as is commonly used by those of ordinary skill in the art. Alternative methods of amplification have been described and can also be employed as long as the BRCA2 locus amplified by PCR using primers of the invention is similarly amplified by the alternative means. Such alternative amplification systems include but are not limited to self-sustained sequence replication, which begins with a short sequence of RNA of interest and a T7 promoter. Reverse transcriptase copies the RNA into cDNA and degrades the RNA, followed by reverse transcriptase polymerizing a second strand of DNA. Another nucleic acid amplification technique is nucleic acid sequence-based amplification (NASBA) which uses reverse transcription and T7 RNA polymerase and incorporates two primers to target its cycling scheme. NASBA can begin with either DNA or RNA and finish with either, and amplifies to 10^8 copies within 60 to 90 minutes. Alternatively, nucleic acid can be amplified by ligation activated transcription (LAT). LAT works from a single-stranded template with a single primer that is partially single-stranded and partially double-stranded. Amplification is initiated by ligating a cDNA to the promoter oligonucleotide and within a few hours, amplification is 10^8 to 10^9 fold. The QB replicase system can be utilized by attaching an RNA sequence called MDV-1 to RNA complementary to a DNA sequence of interest. Upon mixing with a sample, the hybrid RNA finds its complement among the specimen's mRNAs and binds, activating the replicase to copy the tag-along sequence of interest. Another nucleic acid amplification technique, ligase chain reaction

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