US 20100143916A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2010/0143916 A1**

(43) **Pub. Date: Jun. 10, 2010**

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(54) **OSTEOARTHRITIS BIOMARKERS AND USES THEREOF**

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(21) Appl. No.: **12/554,141**

(22) Filed: **Sep. 4, 2009**

Related U.S. Application Data

(62) Division of application No. 10/915,680, filed on Aug. 9, 2004, now abandoned.

(60) Provisional application No. 60/493,607, filed on Aug. 8, 2003, provisional application No. 60/516,823, filed on Nov. 3, 2003.

Publication Classification

(51) **Int. Cl.**
C12Q 1/68 (2006.01)

(52) **U.S. Cl.** **435/6**

(57) **ABSTRACT**

The invention relates to the identification and selection of novel biomarkers and the identification and selection of novel biomarker combinations which are differentially expressed in osteoarthritis and/or in a particular stage of osteoarthritis, as well as a means of selecting the novel biomarker combinations. The measurement of expression of the products of the biomarkers and combinations of biomarkers of the invention demonstrates particular advantage in one or more of the following: (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). As would be understood, in order to measure the products of biomarkers of the invention, polynucleotides and proteins which specifically and/or selectively hybridize to the products of the biomarkers of the invention are also encompassed within the scope of the invention as are kits containing said polynucleotides and proteins for use in (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). Further encompassed by the invention is the use of the polynucleotides and proteins which specifically and/or selectively hybridize to the product of the biomarkers of the invention to monitor disease progression in an individual and to monitor the efficacy of therapeutic regimens. The invention also provides for methods of using the products of the biomarkers of the invention in the identification of novel therapeutic targets for osteoarthritis. The invention also provides for methods of using the products of the biomarkers of the invention in the identification of compounds that bind and/or modulate the activity of the genes of the invention. The compounds identified via such methods are useful for the development of assays to study osteoarthritis and osteoarthritis progression. Further, the compounds identified via such methods are useful as lead compounds in the development of prophylactic and therapeutic compositions for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof.

	Gene Name	Abbr. Gene Name	Gene Accession Numbers and UniGene Accession Numbers	Protein Accession Number	p Values or T-test Values demonstrating statistical significance
1	Beta-2-microglobulin	B2M	NM_004048; HS.48516	NP_004039	0.01
2	Alpha glucosidase II alpha subunit	G2AN	NM_014610; D42041; HS.76847	NP_055425	<0.001
3	interleukin 13 receptor alpha 1	IL13RA1	NM_001560; HS.285115	NP_001551	0.014
4	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	IKBKAP	NM_003640; BC033094; HS.31323	NP_003631	0.002
5	Tumor necrosis factor alpha-induced protein 6	TNFAIP 6	NM_007115; HS.29352	NP_009046	0.003
6	WD repeat domain 9	WDR9	BC047058; NM_018963; HS.225674	Q9NSI6	0.016
7	Nedd-4-like ubiquitin-protein ligase	WWP2	NM_007014; BC013645; HS.333382	NP_008945	0.012
8	B-cell CLL/lymphoma 6	BCL6	NM_138931; HS.155024	NP_620309	0.043
9	Complement component 1, q subcomponent, receptor 1	C1QR1	NM_012072; HS.97199	NP_036204	0.048
10	Cyclin C	CCNC	NM_005190; BC050726; HS.118442	NP_005181	0.028
11	Chromosome 6 open reading frame 151	FLJ3223 4	NM_152551; HS.13366	NP_689764	0.041
12	Heat shock 90kDa protein 1, alpha	HSPCA	NM_005348; BC017233; HS.356531	NP_005339	0.004

FIGURE 1

	Gene Name	Abbr. Gene Name	Gene Accession Numbers and UniGene Accession Numbers	Protein Accession Number	p Values or T-test Values demonstrating statistical significance
13	Laminin, gamma 1	LAMC1	NM_002293; HS.432855	NP_002284	0.009
14	PABP-interacting protein 2	PAIP2	NM_016480; BC048106; HS.396644	NP_057564	0.010
15	Interferon Regulatory Factor 1	IRF1	NM_002198; Hs.80645	NP_002189;	0.01 (t-test)
16	Nuclear receptor coactivator 1	NCOA1	NM_003743; Hs.386092	NP_003734	0.017 (t-test)
17	Homo sapiens chloride intracellular channel 4	CLIC4	NM_013943; Hs.25035	NP_039234	0.044 (t-test)
18	ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1)	ABCA1	NM_005502; Hs.147259	NP_005493	0.022 (t-test)
19	ATP-binding cassette, sub-family G (WHITE), member 1	ABCG1	NM_004915; Hs.369055	NP_004906	0.004 (t-test)

FIGURE 1 (Cont'd)

	Gene Name	Abbreviated Gene Name	Gene Accession Numbers and UniGene Accession Numbers	Protein Accession Number	P Values Demonstrating Statistical Significance
1	Tumor necrosis factor alpha-induced protein 6	TNFAIP6	NM_007115; HS.29352	NP_009046	0.023
2	WD repeat domain 9	WDR9	BC047058; NM_018963; HS.225674	Q9NSI6	0.016
3	Alpha glucosidase II alpha subunit	G2AN	NM_014610; D42041; HS.76847	NP_055425	<0.001
4	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	IKBKAP	NM_003640; BC033094; HS.31323	NP_003631	<0.001

FIGURE 2

	Gene Name	Abbrev. Gene Name	Gene Accession Numbers and UniGene Accession Numbers	Protein Accession Number	P Values demonstrating Statistical Significance
1	Zinc finger RNA binding protein	ZFR	NM_016107; HS.173518	NP_057191	<0.001
2	WD repeat domain 9	WDR9	BC047058; NM_018963; HS.225674	Q9NSI6	0.033

FIGURE 3

	Gene Name	Abbreviated Gene Name	Gene Accession Numbers and UniGene Accession Numbers	Protein Accession Number	P Value Demonstrating Statistical Significance
1	Period 1 (Drosophila)	PER1	NM_002616; HS.68398	NP_002607	0.046
2	EBNA1 binding protein 2	EBNA1BP2	NM_006824; HS.346868	NP_006815	0.012
3	Chromosome 6 open reading frame 151	FLJ32234	NM_152551; HS.13366	NP_689764	0.009
4	Laminin, gamma 1	LAMC1	NM_002293; HS.432855	NP_002284	0.026

FIGURE 4

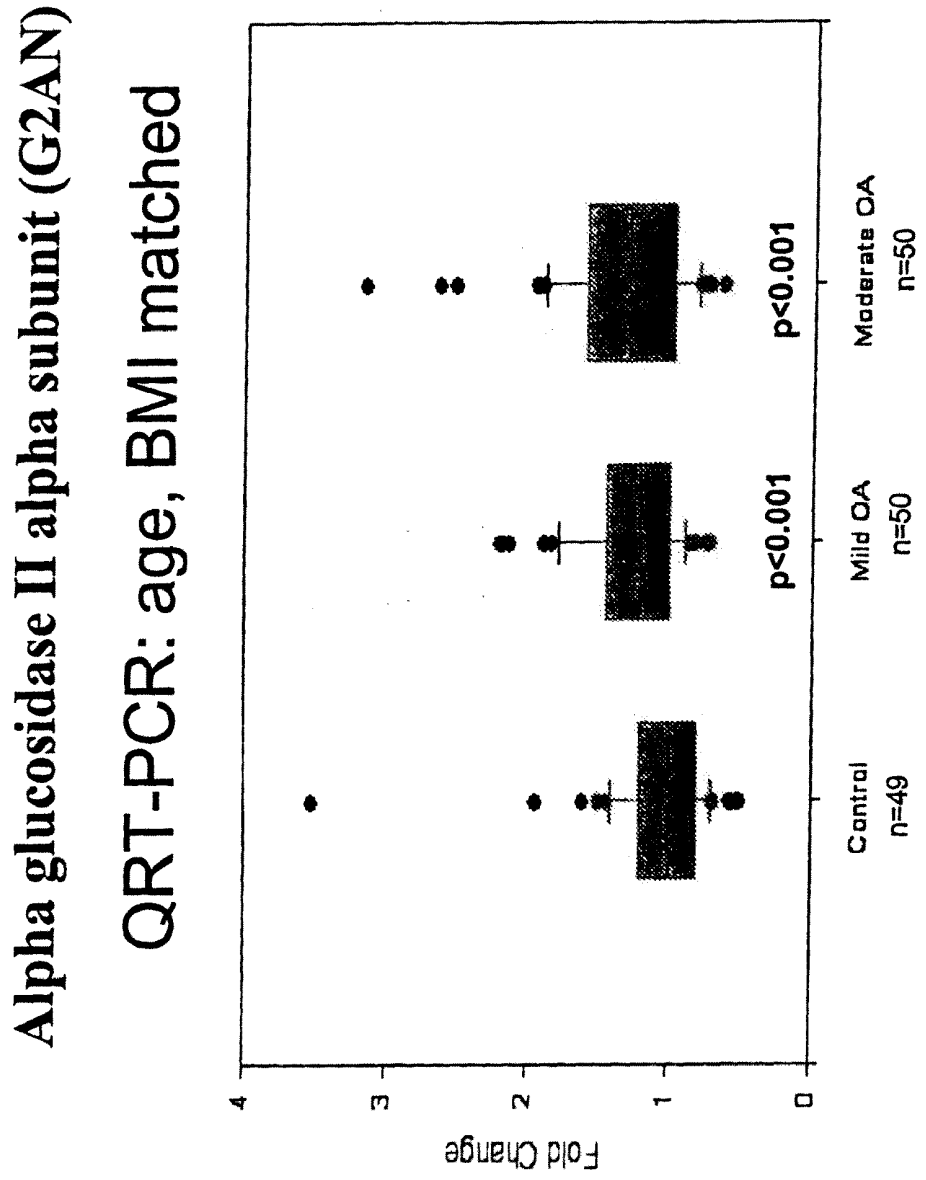
Alpha glucosidase II alpha subunit (G2AN)
QRT-PCR: age, BMI matched
Fold Change
0
1
2
3
4
Control
n=49
Mild OA
n=50
Moderate OA
n=50
p<0.001
p<0.001

FIGURE 5

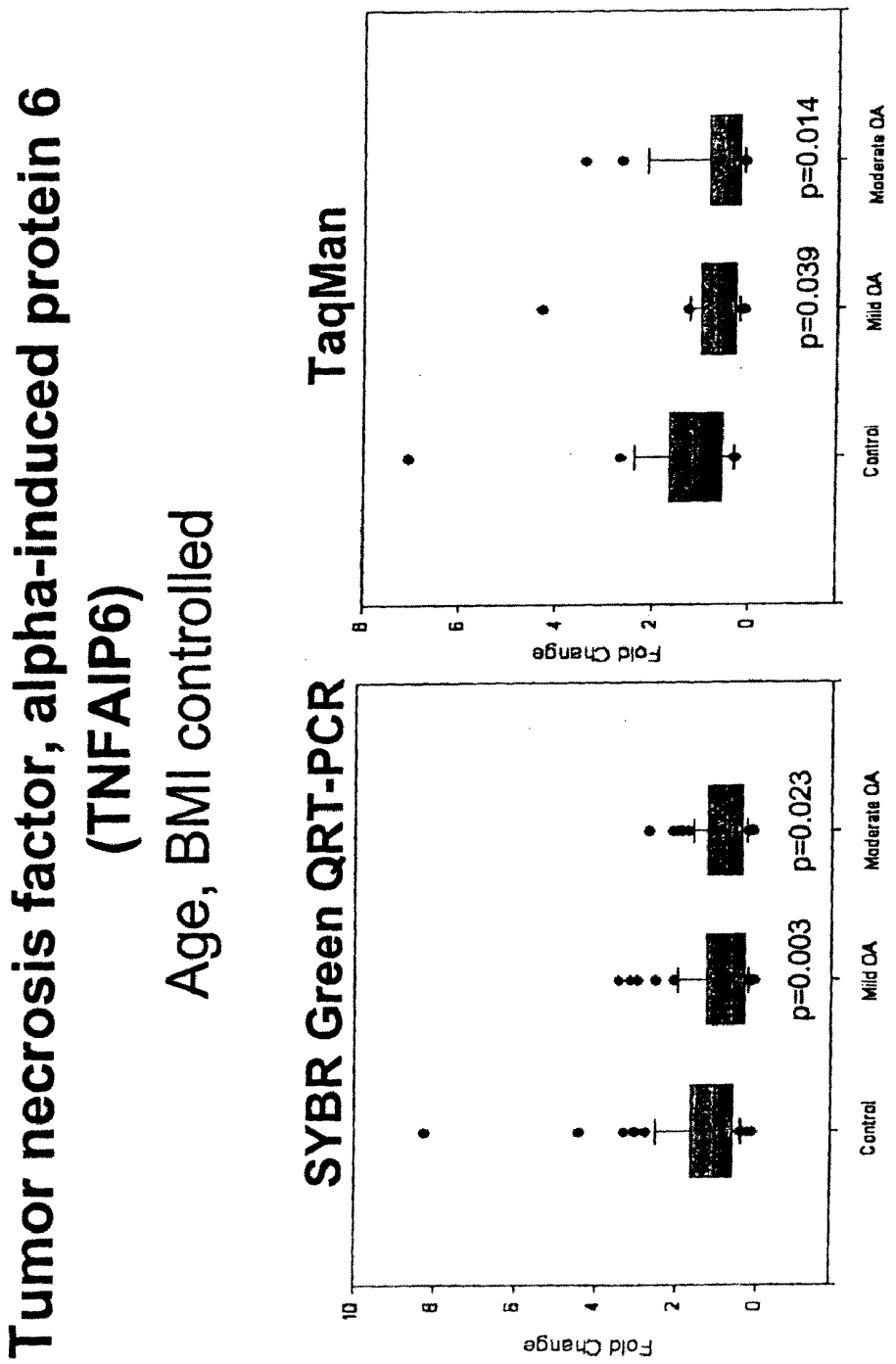
Tumor necrosis factor, alpha-induced protein 6
(TNFAIP6)
Age, BMI controlled
SYBR Green QRT-PCR
TaqMan
Fold Change
Control
Mild OA
Moderate OA
p=0.003
p=0.023
p=0.039
p=0.014

FIGURE 6

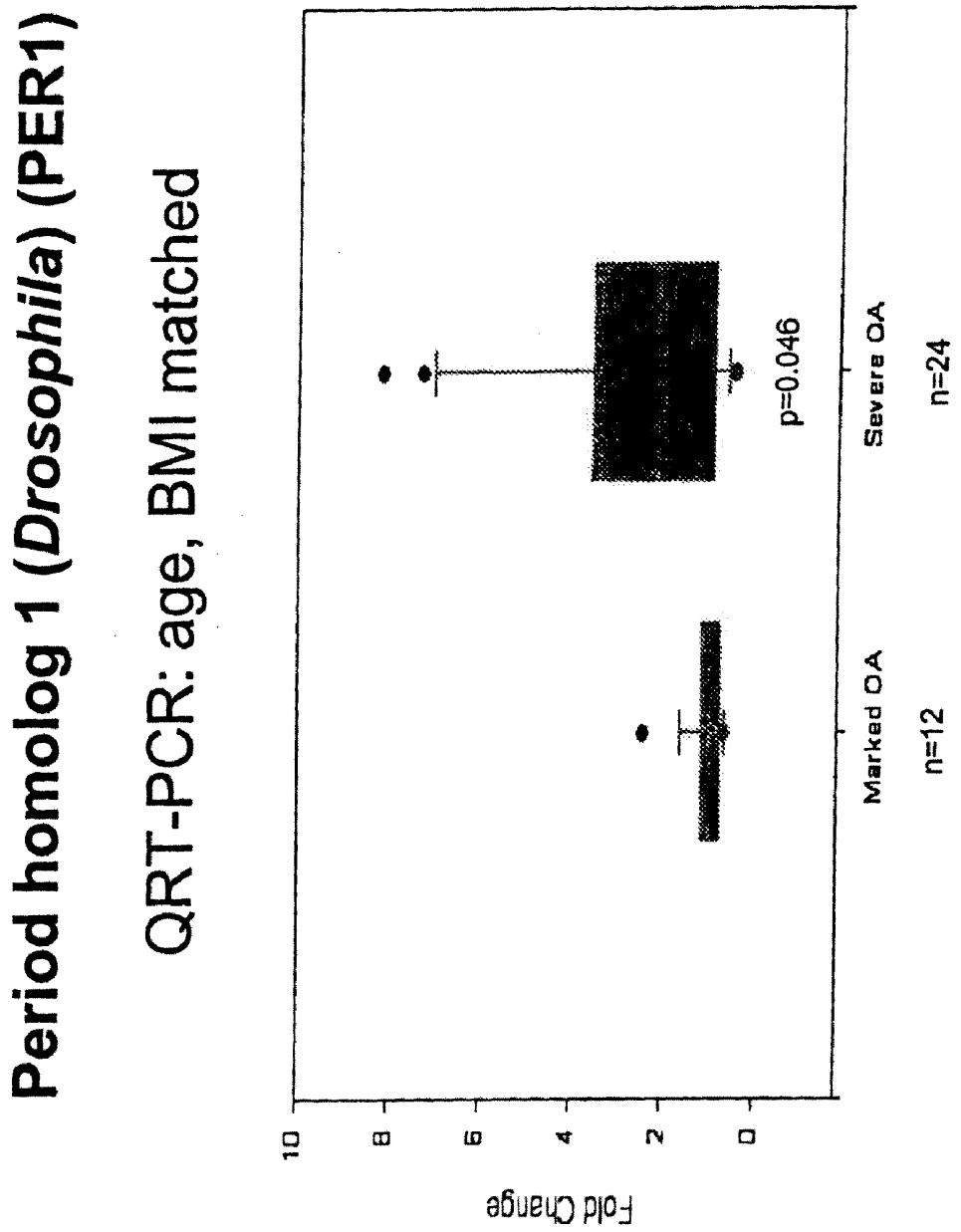
Period homolog 1 (Drosophila) (PER1)
QRT-PCR: age, BMI matched
Fold Change
10
8
6
4
2
0
p=0.046
Marked OA
Severe OA
n=12
n=24

FIGURE 7

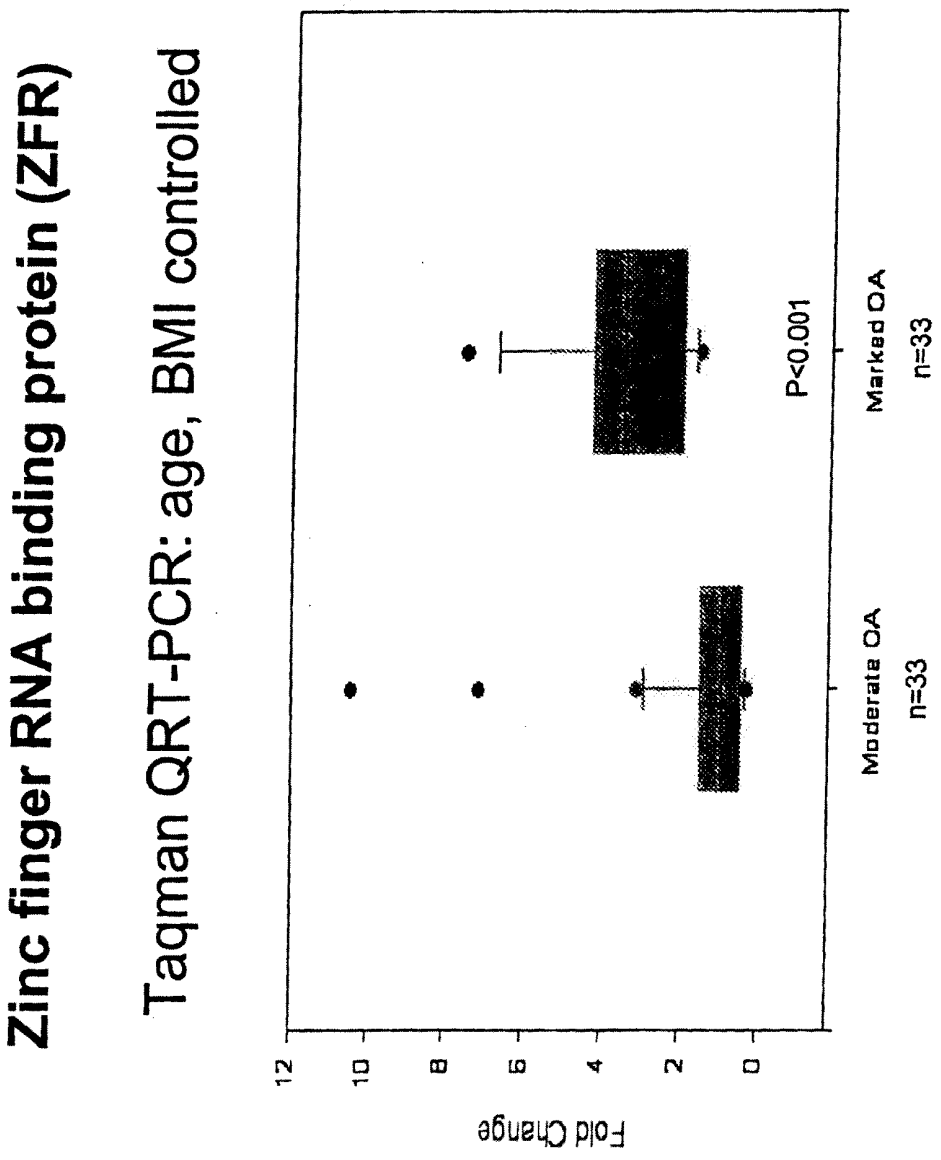
Zinc finger RNA binding protein (ZFR)
Taqman QRT-PCR: age, BMI controlled
Fold Change
12
10
8
6
4
2
0
P<0.001
Moderate OA
n=33
Marked OA
n=33

FIGURE 8

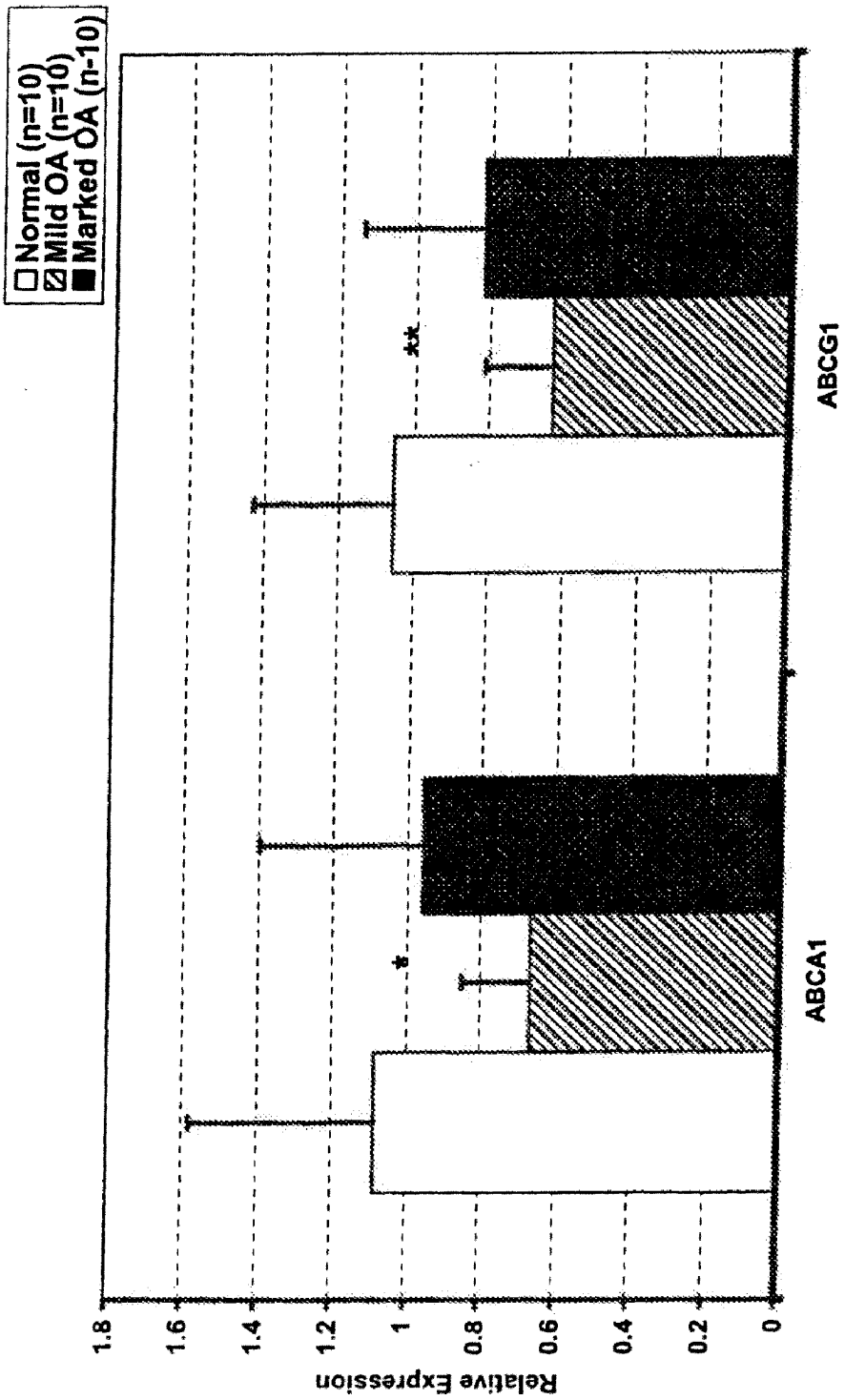
Normal (n=10)
Mild OA (n=10)
Marked OA (n-10)
Relative Expression
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ABCA1
ABCG1
*
**

FIGURE 9

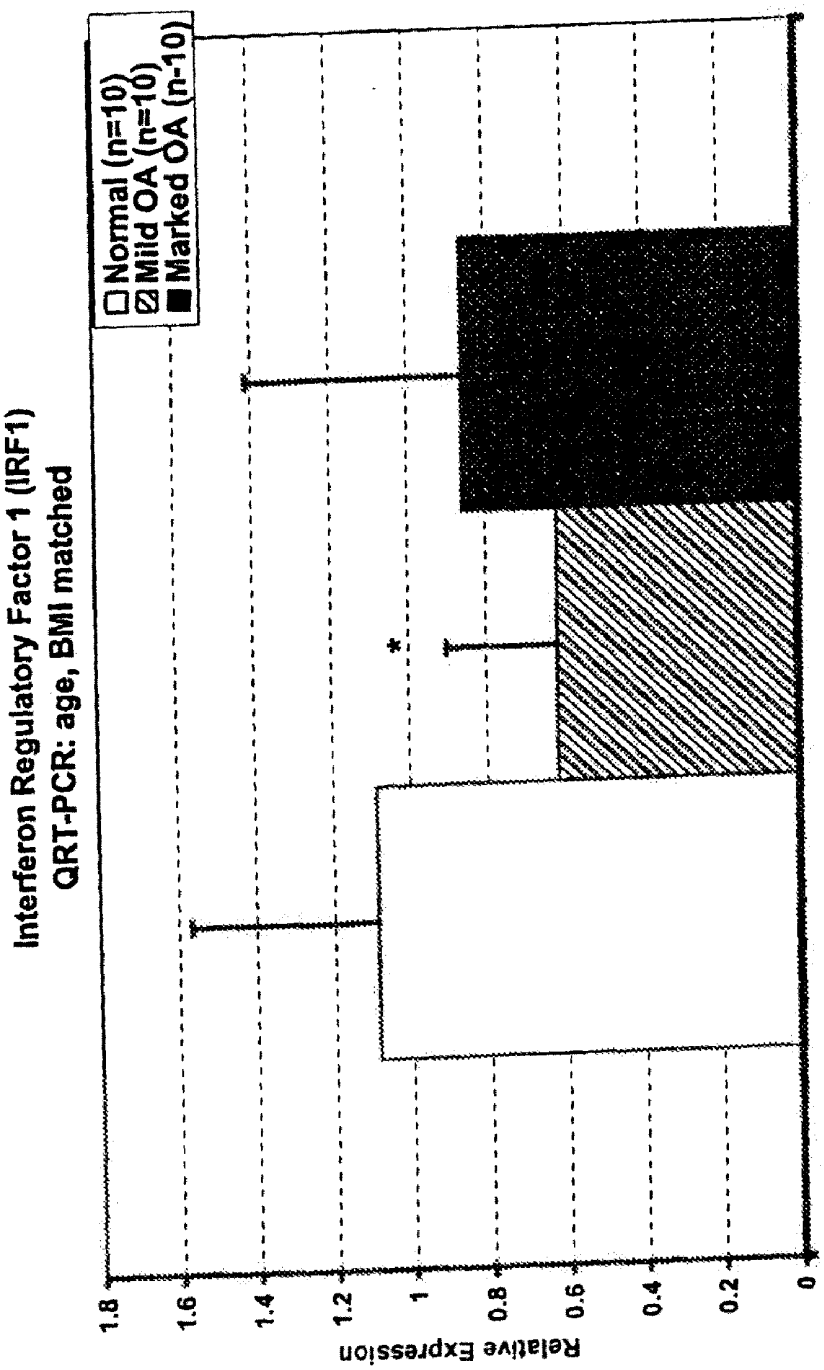
Interferon Regulatory Factor 1 (IRF1)
QRT-PCR: age, BMI matched
Normal (n=10)
Mild OA (n=10)
Marked OA (n-10)
Relative Expression
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
IRF1

FIGURE 10

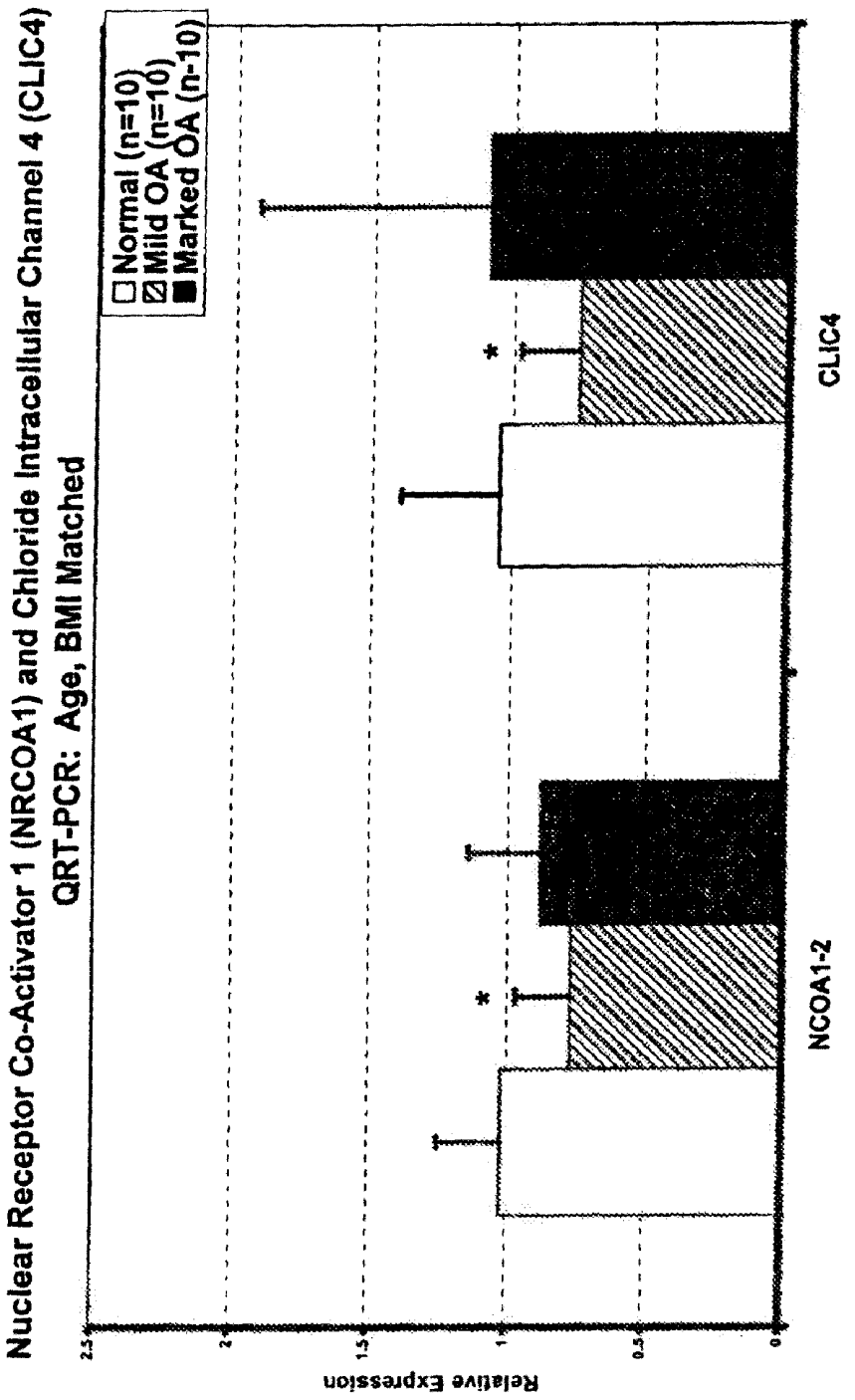
Nuclear Receptor Co-Activator 1 (NRCOA1) and Chloride Intracellular Channel 4 (CLIC4)
QRT-PCR: Age, BMI Matched
Normal (n=10)
Mild OA (n=10)
Marked OA (n-10)
Relative Expression
2.5
2
1.5
1
0.5
0
*
*
NCOA1-2
CLIC4

FIGURE 11

OSTEOARTHRITIS BIOMARKERS AND USES THEREOF

[0001] This application is a divisional of U.S. application Ser. No. 10/915,680, filed Aug. 9, 2004, now abandoned, which claims priority benefit under 35 U.S.C. §119 to U.S. provisional application Ser. No. 60/493,607, filed Aug. 8, 2003 and U.S. provisional application Ser. No. 60/516,823, filed Nov. 3, 2003, each of which is incorporated herein by reference in its entirety.

1. FIELD OF THE INVENTION

[0002] The invention relates to the identification and selection of novel biomarkers and the identification and selection of novel biomarker combinations which are differentially expressed in osteoarthritis and/or in a particular stage of osteoarthritis, as well as a means of selecting the novel biomarker combinations. The measurement of expression of the products of the biomarkers and combinations of biomarkers of the invention demonstrates particular advantage in one or more of the following: (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). As would be understood, in order to measure the products of biomarkers of the invention, polynucleotides and proteins which specifically and/or selectively hybridize to the products of the biomarkers of the invention are also encompassed within the scope of the invention as are kits containing said polynucleotides and proteins for use in (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). Further encompassed by the invention is the use of the polynucleotides and proteins which specifically and/or selectively hybridize to the product of the biomarkers of the invention to monitor disease progression in an individual and to monitor the efficacy of therapeutic regimens. The invention also provides for methods of using the products of the biomarkers of the invention in the identification of novel therapeutic targets for osteoarthritis. The invention also provides for methods of using the products of the biomarkers of the invention in the identification of compounds that bind and/or modulate the activity of the genes of the invention. The compounds identified via such methods are useful for the development of assays to study osteoarthritis and osteoarthritis progression. Further, the compounds identified via such methods are useful as lead compounds in the development of prophylactic and therapeutic compositions for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof.

2. BACKGROUND OF THE INVENTION

[0003] Osteoarthritis (OA) is a chronic disease in which the articular cartilage that lies on the ends of bones that forms the articulating surface of the joints gradually degenerates over time. There are many factors that are believed to predispose a patient to osteoarthritis including genetic susceptibility, obesity, accidental or athletic trauma, surgery, drugs and heavy physical demands. Osteoarthritis is thought to be initiated by damage to the cartilage of joints. The two most common injuries to joints are sports-related injuries and long term "repetitive use" joint injuries. Joints most commonly affected by osteoarthritis are the knees, hips and hands. In most cases, due to the essential weight-bearing function of the knees and hips, osteoarthritis in these joints causes much more disability than osteoarthritis of the hands. As cartilage degeneration progresses, secondary changes occur in other tissues in and around joints including bone, muscle, ligaments, menisci and synovium. The net effect of the primary failure of cartilage tissue and secondary damage to other tissues is that the patient experiences pain, swelling, weakness and loss of functional ability in the afflicted joint(s). These symptoms frequently progress to the point that they have a significant impact in terms of lost productivity and or quality of life consequences for the patient.

[0004] Articular cartilage is predominantly composed of chondrocytes, type II collagen, proteoglycans and water. Articular cartilage has no blood or nerve supply and chondrocytes are the only type of cell in this tissue. Chondrocytes are responsible for manufacturing the type II collagen and proteoglycans that form the cartilage matrix. This matrix in turn has physical-chemical properties that allow for saturation of the matrix with water. The net effect of this structural-functional relationship is that articular cartilage has exceptional wear characteristics and allows for almost frictionless movement between the articulating cartilage surfaces. In the absence of osteoarthritis, articular cartilage often provides a lifetime of pain-free weight bearing and unrestricted joint motion even under demanding physical conditions.

[0005] Like all living tissues, articular cartilage is continually undergoing a process of renewal in which "old" cells and matrix components are being removed (catabolic activity) and "new" cells and molecules are being produced (anabolic activity). Relative to most tissues, the rate of anabolic/catabolic turnover in articular cartilage is low. Long-term maintenance of the structural integrity of mature cartilage relies on the proper balance between matrix synthesis and degradation. Chondrocytes maintain matrix equilibrium by responding to chemical and mechanical stimuli from their environment. Appropriate and effective chondrocyte responses to these stimuli are essential for cartilage homeostasis. Disruption of homeostasis through either inadequate anabolic activity or excessive catabolic activity can result in cartilage degradation and osteoarthritis (Adams et al., 1995, Nature 377 Suppl:3-174). Most tissues that are damaged and have increased catabolic activity are able to mount an increased anabolic response that allows for tissue healing. Unfortunately, chondrocytes have very limited ability to up-regulate their anabolic activity and increase the synthesis of proteoglycan and type II collagen in response to damage or loss of cartilage matrix.

[0006] Currently there is no known medical treatment to reverse the effects of this cartilage damage. Rather all current therapies for osteoarthritis are directed towards treating the symptoms. In addition, because of the insidious occurrence and slow progression of osteoarthritis, identification of osteoarthritis is often done at a late stage in disease development rather than early in disease progression when potential treatments would be more likely to be effective. As a result further advances in preventing, modifying or curing the osteoarthritic disease process critically depend on identification of early diagnostic markers of disease so as to allow early intervention.

[0007] "Early stage osteoarthritis" is currently very difficult to diagnose. The physician relies primarily on the patient's history and physical exam to make the diagnosis of

mild osteoarthritis. X-rays do not show the underlying early changes in articular cartilage. Currently there are no recognized biochemical markers used to confirm the diagnosis of early stage osteoarthritis. Symptoms, such as episodic joint pain, are a common manifestation of early osteoarthritis. Joints become tender during an episode, which can last days to weeks and remit spontaneously. These symptoms, however, often do not correlate well with the pathological stages of damage to the cartilage. A more reliable measure of "early stage" osteoarthritis can be obtained by determining the extent of cartilage damage, however there is currently no method for measuring cartilage deterioration which is relatively non-invasive.

[0008] The clinical exam of a joint with "late stage" osteoarthritis reveals tenderness, joint deformity and a loss of mobility. Passive joint movement during examination may elicit crepitus or the grinding of bone-on-bone as the joint moves. X-ray changes are often profound: the joint space may be obliterated and misalignment of the joint can be seen. New bone formation (osteophytes) is prominent. Again, there are no non-invasive methods which can be used to accurately confirm the diagnosis of "late stage osteoarthritis".

[0009] Thus there is a need for a simple non-invasive diagnostic test for detecting the various stages of osteoarthritis, and a prognostic test that effectively monitors a patient's response to therapy.

3. SUMMARY OF THE INVENTION

[0010] The invention relates to the identification and selection of novel biomarkers and the identification and selection of novel biomarker combinations which are differentially expressed in osteoarthritis and/or in a particular stage of osteoarthritis, as well as a means of selecting the novel biomarker combinations. The measurement of expression of the products of the biomarkers and combinations of biomarkers of the invention demonstrates particular advantage in one or more of the following: (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). As would be understood, in order to measure the products of biomarkers of the invention, polynucleotides and proteins which specifically and/or selectively hybridize to the products of the biomarkers of the invention are also encompassed within the scope of the invention as are kits containing said polynucleotides and proteins for use in (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). Further encompassed by the invention is the use of the polynucleotides and proteins which specifically and/or selectively hybridize to the product of the biomarkers of the invention to monitor disease progression in an individual and to monitor the efficacy of therapeutic regimens. The invention also provides for the identification of methods of using the products of the biomarkers of the invention in the identification of novel therapeutic targets for osteoarthritis. The invention also provides for the identification of methods of using the products of the biomarkers of the invention in the identification of compounds that bind and/or modulate the activity of the genes of the invention. The compounds identified via such methods are useful for the development of assays to study osteoarthritis and osteoarthritis progression. Further, the compounds identified via such methods are useful as lead compounds in the development of prophylactic and therapeutic compositions for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof.

[0011] In another embodiment, the method of determining whether a person has OA comprises the steps of (a) isolating total cellular protein from a test individual; (b) generating monoclonal antibodies specific for the polypeptides encoded by one or more biomarkers, or portions thereof, of the invention for use as an antibody target (c) spotting the antibody targets of step (b) to an array; and (d) incubating the total cellular protein from a test individual to said array; and (e) measuring the amount of binding at each unique location on the array; and (f) using the measured amount of binding for input into the equation(s) generated by the mathematical model used to identify the combination in order to determine a diagnosis as defined by the model.

[0012] In one embodiment, the invention provides for an isolated biomarker comprising two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase H alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0013] In one embodiment, the invention provides for an isolated biomarker consisting essentially of 19 genes as set out in FIG. **1**.

[0014] In one embodiment, the invention provides for an isolated biomarker comprising two or more genes selected from the group consisting of the genes Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Alpha glucosidase II alpha subunit and the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, as set out in FIG. **2**.

[0015] In one embodiment, the invention provides for an isolated biomarker comprising the genes Zinc finger RNA binding protein and WD repeat domain 9, as set out in FIG. **3**.

[0016] In one embodiment, the invention provides for an isolated biomarker comprising two or more genes selected from the group consisting of the genes Period 1 (*Drosophila*), EBNA1 binding protein 2, Chromosome 6 open reading frame 151 and Laminin gamma 1, as set out in FIG. **4**.

[0017] In one embodiment, the invention provides for an isolated biomarker comprising one or more polynucleotide sequences from the 5' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cas-

sette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0018] In one embodiment, the invention provides for an isolated biomarker comprising one or more polynucleotide sequences from the 3' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0019] In one embodiment, the invention provides for an isolated biomarker comprising one or more polynucleotide sequences from the internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0020] In another embodiment, the invention provides for an isolated biomarker comprising one or more polynucleotide sequences that are amplified from the 5' region, 3' region or internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0021] In another embodiment, the invention provides for an isolated biomarker comprising one or more polynucleotide sequences that are expression sequence tags from the 5' region, 3' region or internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase H alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0022] In another embodiment, the invention further provides for an isolated biomarker comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0023] In another embodiment, the invention further provides for an isolated biomarker consisting essentially of the polypeptide sequences encoded by the 19 genes, as set out in FIG. **1**.

[0024] In another embodiment, the invention further provides for an isolated biomarker comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9 Alpha glucosidase II alpha subunit and the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, as set out in FIG. **2**.

[0025] In another embodiment, the invention further provides for an isolated biomarker comprising the polypeptide sequences encoded by the genes Zinc finger RNA binding protein and WD repeat domain 9, as set out in FIG. **3**.

[0026] In another embodiment, the invention further provides for an isolated biomarker comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Period 1 (*Drosophila*), EBNA1 binding protein 2, Chromosome 6 open reading frame 151 and Laminin gamma 1, as set out in FIG. **4**.

[0027] In another embodiment, the invention further provides for an isolated biomarker comprising the amino terminal polypeptide sequences encoded by one or more polynucleotide sequences from the 5' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1),

member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0028] In another embodiment, the invention further provides for an isolated biomarker comprising the carboxy terminal polypeptide sequences encoded by one or more polynucleotide sequences from the 3' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0029] In another embodiment, the invention further provides for an isolated biomarker comprising the internal polypeptide region sequences encoded by one or more polynucleotide sequences from the internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0030] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0031] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker consisting essentially of 19 genes as set out in FIG. **1**.

[0032] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising two or more genes selected from the group consisting of the genes Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Alpha glucosidase II alpha subunit and the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, as set out in FIG. **2**.

[0033] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising the genes Zinc finger RNA binding protein and WD repeat domain 9, as set out in FIG. **3**.

[0034] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising two or more genes selected from the group consisting of the genes Period 1 (*Drosophila*), EBNA1 binding protein 2, Chromosome 6 open reading frame 151 and Laminin gamma 1, as set out in FIG. **4**.

[0035] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising one or more polynucleotide sequences from the 5' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0036] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising one or more polynucleotide sequences from the 3' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0037] In another embodiment, the invention further provides for a composition comprising a probe that specifically hybridizes to an isolated biomarker comprising one or more polynucleotide sequences from the internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1

and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0038] In one embodiment, the invention provides for a probe that is single or double stranded RNA or single or double stranded DNA.

[0039] In one embodiment, the invention provides for a composition comprising the gene for the complement component 1q subcomponent receptor 1.

[0040] In one embodiment, the invention provides for a composition comprising the gene for chromosome 6 open reading frame 151.

[0041] In one embodiment, the invention provides for a composition comprising the gene for WD repeat domain 9.

[0042] In one embodiment, the invention provides for a composition comprising the polypeptide encoded by the gene for the complement component 1q subcomponent receptor 1.

[0043] In one embodiment, the invention provides for a composition comprising the polypeptide encoded by the gene for chromosome 6 open reading frame 151.

[0044] In one embodiment, the invention provides for a composition comprising the polypeptide encoded by the gene for WD repeat domain 9.

[0045] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0046] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker consisting essentially of the polypeptide sequences encoded by the 19 genes, as set out in FIG. **1**.

[0047] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9 Alpha glucosidase II alpha subunit and the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, as set out in FIG. **2**.

[0048] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the polypeptide sequences encoded by the genes Zinc finger RNA binding protein and WD repeat domain 9, as set out in FIG. **3**.

[0049] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Period 1 (*Drosophila*), EBNA1 binding protein 2, Chromosome 6 open reading frame 151 and Laminin gamma 1, as set out in FIG. **4**.

[0050] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the amino terminal polypeptide sequences encoded by one or more polynucleotide sequences from the 5' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0051] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the carboxy terminal polypeptide sequences encoded by one or more polynucleotide sequences from the 3' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0052] In one embodiment, the invention provides for a composition comprising a ligand that specifically binds to a polypeptide encoded by a gene of an isolated biomarker comprising the internal polypeptide region sequences encoded by one or more polynucleotide sequences from the internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-

family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**.

[0053] In another embodiment, the invention provides for a ligand that is a monoclonal antibody.

[0054] In another embodiment, the invention provides for a kit comprising an isolated biomarker of one or more of the subject isolated biomarkers described above and packaging means therefore.

[0055] In another embodiment, the invention provides for a microarray comprising an isolated biomarker of one or more of the subject isolated biomarkers, described above, bound to a solid support.

[0056] In another embodiment, the invention provides for a microarray comprising ligands bound to a support, where the ligands specifically bind to one or more of the subject isolated biomarkers, described above.

[0057] In another embodiment, the ligand is a monoclonal antibody.

[0058] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0059] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers consisting essentially of 19 genes as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0060] Another aspect of the invention relates to a method of diagnosing or prognosing mild osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarker comprising two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker of a control, where a difference in expression levels is indicative or predictive of mild osteoarthritis.

[0061] Another aspect of the invention relates to a method of diagnosing or prognosing mild osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarker consisting essentially of the 19 genes as set out in FIG. **1** in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker of a control, where a difference in expression levels is indicative or predictive of mild osteoarthritis.

[0062] Another aspect of the invention relates to a method of diagnosing or prognosing moderate osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising two or more genes selected from the group consisting of the genes Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Alpha glucosidase II alpha subunit and the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, as set out in FIG. **2**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker of a control, where a difference in expression levels is indicative or predictive of moderate osteoarthritis.

[0063] Another aspect of the invention relates to a method of diagnosing or prognosing moderate from marked osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the genes Zinc finger RNA binding protein and WD repeat domain 9, as set out in FIG. **3**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of an individual with moderate osteoarthritis, where a difference in expression levels is indicative or predictive of marked osteoarthritis.

[0064] Another aspect of the invention relates to a method of diagnosing or prognosing marked from severe osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising two or more genes selected from the group consisting of the genes Period 1 (*Drosophila*), EBNA1 binding protein 2, Chromosome 6 open reading frame 151 and Laminin gamma 1, as set out in FIG. **4**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of an individual with marked osteoarthritis, where a difference in expression levels is indicative or predictive of severe osteoarthritis.

[0065] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the polypeptide

sequences encoded by two or more genes selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0066] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers consisting essentially of the polypeptide sequences encoded by the 19 genes, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0067] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9 Alpha glucosidase II alpha subunit and the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, as set out in FIG. **2**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0068] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the polypeptide sequences encoded by the genes Zinc finger RNA binding protein and WD repeat domain 9, as set out in FIG. **3**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0069] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the polypeptide sequences encoded by two or more genes selected from the group consisting of the genes Period 1 (*Drosophila*), EBNA1 binding protein 2, Chromosome 6 open reading frame 151 and Laminin gamma 1, as set out in FIG. **4**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0070] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the amino terminal polypeptide sequences encoded by one or more polynucleotide sequences from the 5' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0071] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the carboxy terminal polypeptide sequences encoded by one or more polynucleotide sequences from the 3' region of a gene selected from the group consisting of the genes Beta-2-microglobulin, Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0072] Another aspect of the invention relates to a method of diagnosing or prognosing osteoarthritis in an individual, comprising the steps of a) determining the level of expression of the isolated biomarkers comprising the internal polypeptide sequences encoded by one or more polynucleotide sequences from the internal coding region of a gene selected from the group consisting of the genes Beta-2-microglobulin,

Alpha glucosidase II alpha subunit, Interleukin 13 receptor alpha 1, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex-associated protein, Tumor necrosis factor alpha-induced protein 6, WD repeat domain 9, Nedd-4-like ubiquitin-protein ligase, B-cell CLL/lymphoma 6, Complement component 1q subcomponent receptor 1, Cyclin C, Chromosome 6 open reading frame 151, Heat shock 90 kDa protein 1 alpha, Laminin gamma 1 and PABP-interacting protein, Interferon Regulatory Factor 1, Nuclear receptor coactivator 1, *Homo sapiens* chloride intracellular channel 4, ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1, as set out in FIG. **1**, in a blood sample of an individual, and b) detecting a difference of the level of expression of the biomarker in the blood sample according to step a) relative to the level of expression of the same biomarker in a blood sample of a control, where a difference in expression levels is indicative or predictive of osteoarthritis.

[0073] Another aspect of the invention relates to a method of diagnosing or prognosing progression of osteoarthritis, comprising the steps of a) determining the level of expression of the isolated biomarker as set forth in FIG. **3** in a blood sample of an individual having moderate osteoarthritis; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with marked osteoarthritis, where a difference in the levels indicates progression of osteoarthritis from moderate to marked osteoarthritis.

[0074] Another aspect of the invention relates to a method of diagnosing or prognosing progression of osteoarthritis, comprising the steps of a) determining the level of expression of the isolated biomarker as set forth in FIG. **4** in a blood sample of an individual having marked osteoarthritis; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with severe osteoarthritis, where a difference in the levels indicates progression of osteoarthritis from marked to severe osteoarthritis.

[0075] Another aspect of the invention relates to a method of diagnosing or prognosing progression of osteoarthritis, comprising the steps of a) determining the level of expression of a biomarker in a blood sample of an individual having moderate osteoarthritis, where the biomarker comprises one of the genes disclosed in FIG. **3**; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with marked osteoarthritis, where a difference in the levels indicates progression of osteoarthritis from moderate to marked osteoarthritis.

[0076] Another aspect of the invention relates to a method of diagnosing or prognosing progression of osteoarthritis, comprising the steps of a) determining the level of expression of a biomarker in a blood sample of an individual having marked osteoarthritis, where the biomarker comprises one of the genes disclosed in FIG. **4**; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with severe osteoarthritis, where a difference in the levels indicates progression of osteoarthritis from marked to severe osteoarthritis.

[0077] Another aspect of the invention relates to a method of diagnosing or prognosing regression of osteoarthritis, comprising the steps of a) determining the level of expression of the isolated biomarker as set forth in FIG. **3** in a blood sample of an individual having marked osteoarthritis; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with moderate osteoarthritis, where a difference in the levels indicates regression of osteoarthritis from marked to moderate osteoarthritis.

[0078] Another aspect of the invention relates to a method of diagnosing or prognosing regression of osteoarthritis, comprising the steps of a) determining the level of expression of the isolated biomarker as set forth in FIG. **4** in a blood sample of an individual having severe osteoarthritis; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with marked osteoarthritis, where a difference in the levels indicates regression of osteoarthritis from severe to marked osteoarthritis.

[0079] Another aspect of the invention relates to a method of diagnosing or prognosing regression of osteoarthritis, comprising the steps of a) determining the level of expression of a biomarker in a blood sample of an individual having marked osteoarthritis, where the biomarker comprises one of the genes disclosed in FIG. **3**; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with moderate osteoarthritis, where a difference in the levels indicates regression of osteoarthritis from marked to moderate osteoarthritis.

[0080] Another aspect of the invention relates to a method of diagnosing or prognosing regression of osteoarthritis, comprising the steps of a) determining the level of expression of a biomarker in a blood sample of an individual having severe osteoarthritis, where the biomarker comprises one of the genes disclosed in FIG. **4**; and b) comparing the level of the step a) with the level of expression of the corresponding isolated biomarker in an individual with marked osteoarthritis, where a difference in the levels indicates regression of osteoarthritis from severe to marked osteoarthritis.

[0081] Another aspect of the invention relates to a method for monitoring efficacy of a drug for treatment of osteoarthritis in a patient, comprising the steps of a) obtaining a blood sample from a patient before treatment and a second blood sample from the patient after the treatment; b) detecting the level of expression of the isolated biomarker as disclosed in FIG. **3** in the first and second blood sample; and comparing the level of expression of the biomarker in the first sample with the second sample, where a differential expression of the level of expression of the biomarker in the first sample as compared with the second sample is indicative of the efficacy of the drug for treatment of osteoarthritis in the patient.

[0082] Another aspect of the invention relates to a method for monitoring efficacy of a drug for treatment of osteoarthritis in a patient, comprising the steps of obtaining a blood sample from a patient before treatment and a second blood sample from the patient after the treatment; detecting the level of expression of the isolated biomarker as disclosed in FIG. **4** in the first and second blood sample; and comparing the level of expression of the biomarker in the first sample with the second sample, where a differential expression of the level of expression of the biomarker in the first sample as compared with the second sample is indicative of the efficacy of the drug for treatment of osteoarthritis in the patient.

[0083] Another aspect of the invention relates to a method for monitoring efficacy of a drug for treatment of osteoarthritis in a patient, comprising the steps of obtaining a blood sample from a patient before treatment and a second blood sample from the patient after the treatment; detecting the level of expression of a biomarker in the first and second blood

sample, wherein the biomarker comprises one of the genes disclosed in FIG. **3**; and comparing the level of expression of the biomarker in the first sample with the second sample, where a differential expression of the level of expression of the biomarker in the first sample as compared with the second sample is indicative of the efficacy of the drug for treatment of osteoarthritis in the patient.

[0084] Another aspect of the invention relates to a method for monitoring efficacy of a drug for treatment of osteoarthritis in a patient, comprising the steps of obtaining a blood sample from a patient before treatment and a second blood sample from the patient after the treatment; detecting the level of expression of a biomarker in the first and second blood sample, wherein the biomarker comprises one of the genes disclosed in FIG. **4**; and comparing the level of expression of the biomarker in the first sample with the second sample, where a differential expression of the level of expression of the biomarker in the first sample as compared with the second sample is indicative of the efficacy of the drug for treatment of osteoarthritis in the patient.

3.1 Definitions

[0085] The following definitions are provided for specific terms which are used in the following written description.

[0086] As used herein, the term "3' end" refers to the end of an mRNA up to the last 1000 nucleotides or ⅓ of the mRNA, where the 3' terminal nucleotide is that terminal nucleotide of the coding or untranslated region that adjoins the poly-A tail, if one is present. That is, the 3' end of an mRNA does not include the poly-A tail, if one is present. The "3' region" of a gene refers to a polynucleotide (double-stranded or single-stranded) located within or at the 3' end of a gene, and includes, but is not limited to, the 3' untranslated region, if that is present, and the 3' protein coding region of a gene. The 3' region is not shorter than 8 nucleotides in length and not longer than 1000 nucleotides in length. Other possible lengths of the 3' region include but are not limited to 10, 20, 25, 50, 100, 200, 400, and 500 nucleotides.

[0087] As used herein, the term "5' end" refers to the end of an mRNA up to the first 1000 nucleotides or ⅓ of the mRNA (where the full length of the mRNA does not include the poly A tail), starting at the first nucleotide of the mRNA. The "5' region" of a gene refers to a polynucleotide (double-stranded or single-stranded) located within or at the 5' end of a gene, and includes, but is not limited to, the 5' untranslated region, if that is present, and the 5' protein coding region of a gene. The 5' region is not shorter than 8 nucleotides in length and not longer than 1000 nucleotides in length. Other possible lengths of the 5' region include but are not limited to 10, 20, 25, 50, 100, 200, 400, and 500 nucleotides.

[0088] As used herein, the term "amplified", when applied to a nucleic acid sequence, refers to a process whereby one or more copies of a particular nucleic acid sequence is generated from a template nucleic acid, preferably by the method of polymerase chain reaction (Mullis and Faloona, 1987, Methods Enzymol. 155:335). "Polymerase chain reaction" or "PCR" refers to an in vitro method for amplifying a specific nucleic acid template sequence. The PCR reaction involves a repetitive series of temperature cycles and is typically performed in a volume of 50-100 μl. The reaction mix comprises dNTPs (each of the four deoxynucleotides dATP, dCTP, dGTP, and dTTP), primers, buffers, DNA polymerase, and nucleic acid template. The PCR reaction comprises providing a set of polynucleotide primers wherein a first primer contains a sequence complementary to a region in one strand of the nucleic acid template sequence and primes the synthesis of a complementary DNA strand, and a second primer contains a sequence complementary to a region in a second strand of the target nucleic acid sequence and primes the synthesis of a complementary DNA strand, and amplifying the nucleic acid template sequence employing a nucleic acid polymerase as a template-dependent polymerizing agent under conditions which are permissive for PCR cycling steps of (i) annealing of primers required for amplification to a target nucleic acid sequence contained within the template sequence, (ii) extending the primers wherein the nucleic acid polymerase synthesizes a primer extension product. "A set of polynucleotide primers" or "a set of PCR primers" can comprise two, three, four or more primers. In one embodiment, an exo-Pfu DNA polymerase is used to amplify a nucleic acid template in PCR reaction. Other methods of amplification include, but are not limited to, ligase chain reaction (LCR), polynucleotide-specific based amplification (NSBA), or any other method known in the art.

[0089] As used herein, the term "amino terminal" region of a polypeptide refers to the polypeptide sequences encoded by polynucleotide sequences (double-stranded or single-stranded) located within or at the 5' end of a gene, and includes, but is not limited to, the 5' protein coding region of a gene. As used herein, the term "amino terminal" region refers to the amino terminal end of a polypeptide up to the first 300 amino acids or ⅓ of the polypeptide, starting at the first amino acid of the polypeptide. The "amino terminal" region of a polypeptide is not shorter than 3 amino acids in length and not longer than 350 amino acids in length. Other possible lengths of the "amino terminal" region of a polypeptide include but are not limited to 5, 10, 20, 25, 50, 100 and 200 amino acids.

[0090] As used herein, the term "analog" in the context of proteinaceous agent (e.g., proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that possesses a similar or identical function as a second proteinaceous agent but does not necessarily comprise a similar or identical amino acid sequence of the second proteinaceous agent, or possess a similar or identical structure of the second proteinaceous agent. A proteinaceous agent that has a similar amino acid sequence refers to a second proteinaceous agent that satisfies at least one of the following: (a) a proteinaceous agent having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second proteinaceous agent; (b) a proteinaceous agent encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second proteinaceous agent of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues; and (c) a proteinaceous agent encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at

least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second proteinaceous agent. A proteinaceous agent with similar structure to a second proteinaceous agent refers to a proteinaceous agent that has a similar secondary, tertiary or quaternary structure to the second proteinaceous agent. The structure of a proteinaceous agent can be determined by methods known to those skilled in the art, including but not limited to, peptide sequencing, X-ray crystallography, nuclear magnetic resonance, circular dichroism, and crystallographic electron microscopy.

[0091] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity=number of identical overlapping positions/total number of positions.times.100%). In one embodiment, the two sequences are the same length.

[0092] The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, e.g., for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, e.g., to score-50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., of XBLAST and NBLAST) can be used (see, e.g., the NCBI website). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0093] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0094] As used herein, the term "analog" in the context of a non-proteinaceous analog refers to a second organic or inorganic molecule which possess a similar or identical function as a first organic or inorganic molecule and is structurally similar to the first organic or inorganic molecule.

[0095] As used herein, the terms "attaching" and "spotting" refer to a process of depositing a nucleic acid onto a solid substrate to form a nucleic acid array such that the nucleic acid is stably bound to the solid substrate via covalent bonds, hydrogen bonds or ionic interactions.

[0096] As used herein, the term "biomarker" refers to a gene that is differentially regulated as between OA and non OA, and/or as between one or more stages of OA and one or more other stages of OA.

[0097] As used herein, the term "blood nucleic acid sample" refers to nucleic acids derived from blood and can include nucleic acids derived from whole blood, centrifuged lysed blood, serum free whole blood or fractionated blood including peripheral blood leukocytes (PBLs) or other fractions of blood as described herein. By whole blood is meant unfractionated blood, for example, a drop of blood. By centrifuged lysed blood or 'lysed blood' is meant whole blood that is mixed with lysis buffer and centrifuged as described herein (see Example 2). By serum free blood is meant whole blood wherein the serum or plasma is removed by centrifugation as described herein (see Example 2). Preferably, a blood nucleic acid sample is whole blood or centrifuged lysed blood and is total RNA, mRNA or is a nucleic acid corresponding to mRNA, for example, cDNA isolated from said blood. A nucleic acid sample can also include a PCR product derived from total RNA, mRNA or cDNA.

[0098] As used herein, the term "carboxy terminal" region of a polypeptide refers to the polypeptide sequences encoded by polynucleotide sequences (double-stranded or single-stranded) located within or at the 3' end of a gene, and includes, but is not limited to, the 3' protein coding region of a gene. As used herein, the "carboxy terminal" region refers to the carboxy terminal end of a polypeptide up to 300 amino acids or ⅓ of the polypeptide from the last amino acid of the polypeptide. The "3' end" does not include the polyA tail, if one is present. The "carboxy terminal" region of a polypeptide is not shorter than 3 amino acids in length and not longer than 350 amino acids in length. Other possible lengths of the "carboxy terminal" region of a polypeptide include, but are not limited to, 5, 10, 20, 25, 50, 100 and 200 amino acids.

[0099] As used herein, "cartilage" or "articular cartilage" refers to elastic, translucent connective tissue in mammals, including human and other species. Cartilage is composed predominantly of chondrocytes, type II collagen, small amounts of other collagen types, other noncollagenous proteins, proteoglycans and water, and is usually surrounded by a perichondrium, made up of fibroblasts, in a matrix of type I and type II collagen as well as other proteoglycans. Although most cartilage becomes bone upon maturation, some cartilage remains in its original form in locations such as the nose, ears, knees, and other joints. The cartilage has no blood or nerve supply and chondrocytes are the only type of cell in this tissue.

[0100] As used herein, the term "cartilage nucleic acid sample" refers to nucleic acids derived from cartilage. Preferably, a cartilage nucleic acid sample is total RNA, mRNA or is a nucleic acid corresponding to RNA, for example, cDNA.

A cartilage nucleic acid sample can also include a PCR product derived from total RNA, mRNA or cDNA.

[0101] As used herein, "chondrocyte" refers to cells from cartilage.

[0102] A "coding region" refers to a the region of DNA which is expressed as RNA RNA.

[0103] As used herein, the terms "compound" and "agent" are used interchangeably.

[0104] As used herein, the term "control" or "control sample" in the context of diagnosing osteoarthritis, determining the stage of osteoarthritis, or differentiating between stages of osteoarthritis refers to one or more samples isolated from an individual or group of individuals who are classified as not having osteoarthritis (ie "normal"). The term control or control sample can also refer to the compilation of data derived from samples of one or more individuals classified as not having osteoarthritis (ie "normal"). As used herein, the term "control" in the context of screening for a prophylactic or therapeutic agent refers to a standard or reference for an assay or methodology to which other conditions can be compared.

[0105] As used herein, the term "derivative" in the context of proteinaceous agent (e.g., proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions, and/or additions. The term "derivative" as used herein also refers to a proteinaceous agent which has been modified, i.e., by the covalent attachment of any type of molecule to the proteinaceous agent. For example, but not by way of limitation, an antibody may be modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a proteinaceous agent may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a proteinaceous agent may contain one or more non-classical amino acids. A derivative of a proteinaceous agent possesses a similar or identical function as the proteinaceous agent from which it was derived.

[0106] As used herein, the term "derivative" in the context of a non-proteinaceous derivative refers to a second organic or inorganic molecule that is formed based upon the structure of a first organic or inorganic molecule. A derivative of an organic molecule includes, but is not limited to, a molecule modified, e.g., by the addition or deletion of a hydroxyl, methyl, ethyl, carboxyl or amine group. An organic molecule may also be esterified, alkylated and/or phosphorylated.

[0107] As used herein "Diagnosis of OA" or "OA diagnosis", according to one aspect of the invention refers to a process of determining if an individual is afflicted with OA. In a specific embodiment, "diagnosis of OA" or "OA diagnosis" refers to a determination as between two options, that an individual has OA or that an individual does not have OA. In another specific embodiment, "diagnosis" refers to a determination as between three options, an individual has OA, an individual does not have OA, and it cannot be determined with sufficient degree of certainty whether an individual has OA or does not have OA. "Diagnosis of whether an individual has one of two stages of OA" or "differentiating between stages of OA" refers to a process of determining whether an individual has one of two stages of OA. (e.g. mild v. control (e.g. non OA/normal); moderate v. mild; moderate v. severe; marked v. severe etc. In another specific embodiment, "diagnosis" refers to a determination as between three options, a diagnosis as to one of two stages of OA as described above or a third possibility wherein it cannot be determined with sufficient degree of certainty whether an individual has either of the two stages referred to. "Diagnosis of a stage of OA" or "OA staging", according to the invention refers to a arriving at a decision of whether an individual is afflicted with a particular stage or grade of OA. In a specific embodiment, "OA staging" or "diagnosis of a stage of OA" refers to a determination as between two options, that an individual has one stage of OA or that an individual does not have OA, or any other stage of OA. In another specific embodiment, "OA staging" or "diagnosis of a stage of OA" refers to a determination as between three options, an individual has a specific stage of OA, an individual does not OA and does not have any other stage of OA, or it cannot be determined with sufficient degree of certainty whether an individual has a specific stage of OA or does not have the specific stage of OA. As would be understood by a person skilled in the art, in this context a "sufficient degree of certainty" depends upon the medical requirements for both the sensitivity and specificity of the diagnosis. More particularly the sufficient degree of certainty includes greater than 50% sensitivity and/or specificity, greater than 60% sensitivity and/or specificity, greater than 70% sensitivity and/or specificity, greater than 80% sensitivity and/or specificity, greater than 90% sensitivity and/or specificity and 100% sensitivity and/or specificity.

[0108] As used herein, the term "differential expression" refers to a difference in the level of expression of the RNA and/or protein products of one or more biomarkers of the invention, as measured by the amount or level of RNA, including mRNA, and/or one or more spliced variants of mRNA of the biomarker in one sample as compared with the level of expression of the same one or more biomarkers of the invention as measured by the amount or level of RNA, including mRNA and/or one or more spliced variants of mRNA in a second sample. "Differentially expressed" can also include a measurement of the protein, or one or more protein variants encoded by the biomarker of the invention in a sample or population of samples as compared with the amount or level of protein expression, including one or more protein variants of the biomarker or biomarkers of the invention. Differential expression can be determined as described herein and as would be understood by a person skilled in the art. The term "differentially expressed" or "changes in the level of expression" refers to an increase or decrease in the measurable expression level of a given biomarker as measured by the amount of RNA and/or the amount of protein in a sample as compared with the measurable expression level of a given biomarker a second sample. The term "differentially expressed" or "changes in the level of expression" can also refer to an increase or decrease in the measurable expression level of a given biomarker in a population of samples as compared with the measurable expression level of a biomarker in a second population of samples. As used herein, "differentially expressed" when referring to a single sample can be measured using the ratio of the level of expression of a given biomarker in said sample as compared with the mean expression level of the given biomarker of a control population wherein the ratio is not equal to 1.0. Differentially expressed can also be used to include comparing a first population of samples as compared with a second population of

samples or a single sample to a population of samples using either a ratio of the level of expression or using p-value. When using p-value, a nucleic acid transcript including hnRNA and mRNA is identified as being differentially expressed as between a first and second population when the p-value is less than 0.1. More preferably the p-value is less than 0.05. Even more preferably the p-value is less than 0.01. More preferably still the p-value is less than 0.005. Most preferably the p-value is less than 0.001. When determining differentially expression on the basis of the ratio of the level of expression, an RNA or protein is differentially expressed if the ratio of the level of expression in a first sample as compared with a second sample is greater than or less than 1.0. For example, a ratio of greater than 1.2, 1.5, 1.7, 2, 3, 4, 10, 20 or a ratio less than 1, for example 0.8, 0.6, 0.4, 0.2, 0.1. 0.05. In another embodiment of the invention a nucleic acid transcript including hnRNA and mRNA is differentially expressed if the ratio of the mean of the level of expression of a first population as compared with the mean level of expression of the second population is greater than or less than 1.0 For example, a ratio of greater than 1.2, 1.5, 1.7, 2, 3, 4, 10, 20 or a ratio less than 1, for example 0.8, 0.6, 0.4, 0.2, 0.1. 0.05. In another embodiment of the invention a nucleic acid transcript including hnRNA, and mRNA is differentially expressed if the ratio of its level of expression in a first sample as compared with the mean of the second population is greater than or less than 1.0 and includes for example, a ratio of greater than 1.2, 1.5, 1.7, 2, 3, 4, 10, 20, or a ratio less than 1, for example 0.8, 0.6, 0.4, 0.2, 0.1. 0.05."Differentially increased expression" refers to 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, or more, relative to a standard, such as the mean of the expression level of the second population. "Differentially decreased expression" refers to less than 1.0 fold, 0.8 fold, 0.6 fold, 0.4 fold, 0.2 fold, 0.1 fold or less, relative to a standard, such as the mean of the expression level of the second population.

[0109] As used herein, the term "drug efficacy" refers to the effectiveness of a drug. "Drug efficacy" is usually measured by the clinical response of the patient who has been or is being treated with a drug. A drug is considered to have a high degree of efficacy, if it achieves desired clinical results, for example, the reduction of the symptoms of osteoarthritis or the prevention of osteoarthritis progression as described in the present specification. The amount of drug absorbed may be used to predict a patient's response. A general rule is that as the dose of a drug is increased, a greater effect is seen in the patient until a maximum desired effect is reached. If more drug is administered after the maximum point is reached, the side effects will normally increase.

[0110] As used herein, the term "effective amount" refers to the amount of a compound which is sufficient to reduce or ameliorate the progression, severity and/or duration of osteoarthritis or one or more symptoms thereof, prevent the development, recurrence or onset of osteoarthritis or one or more symptoms thereof, prevent the advancement of osteoarthritis or one or more symptoms thereof, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[0111] As used herein, the term "fragment" in the context of a proteinaceous agent refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of another polypeptide or a protein. In a specific embodiment, a fragment of a protein or polypeptide retains at least one function of the protein or polypeptide. In another embodiment, a fragment of a protein or polypeptide retains at least two, three, four, or five functions of the protein or polypeptide. Preferably, a fragment of an antibody retains the ability to immunospecifically bind to an antigen.

[0112] As used herein, the term "fusion protein" refers to a polypeptide that comprises an amino acid sequence of a first protein or polypeptide or functional fragment, analog or derivative thereof, and an amino acid sequence of a heterologous protein, polypeptide, or peptide (i.e., a second protein or polypeptide or fragment, analog or derivative thereof different than the first protein or fragment, analog or derivative thereof). In one embodiment, a fusion protein comprises a prophylactic or therapeutic agent fused to a heterologous protein, polypeptide or peptide. In accordance with this embodiment, the heterologous protein, polypeptide or peptide may or may not be a different type of prophylactic or therapeutic agent.

[0113] As used herein, the terms "gene expression pattern", "gene expression profile" and "nucleic acid array expression profile" are used interchangeably and comprise the pattern of hybridization of a plurality of target nucleic acid sequences hybridized to a plurality of nucleic acid probes on an array as compared with a control.

[0114] As used herein, the terms "hybridizing to" and "hybridization" refer to the sequence specific non-covalent binding interactions with a complementary nucleic acid, for example, interactions between a target nucleic acid sequence and a nucleic acid member on an array.

[0115] As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

[0116] As used herein, the term "in combination" refers to the use of more than one therapy (e.g., more than one prophylactic agent and/or therapeutic agent). The use of the term "in combination" does not restrict the order in which therapies (e.g., prophylactic and/or therapeutic agents) are administered to a subject. A first therapy (e.g., a first prophylactic or therapeutic agent) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4

hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) to a subject.

[0117] As used herein, the term "indicative of disease" refers to an expression pattern which is diagnostic of disease such that the expression pattern is found significantly more often in patients with a disease than in patients without the disease (as determined using routine statistical methods setting confidence levels at a minimum of 95%). Preferably, an expression pattern which is indicative of disease is found in at least 60% of patients who have the disease and is found in less than 10% of patients who do not have the disease. More preferably, an expression pattern which is indicative of disease is found in at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more in patients who have the disease and is found in less than 10%, less than 8%, less than 5%, less than 2.5%, or less than 1% of patients who do not have the disease.

[0118] As used herein, the term "internal coding region" of a gene refers to a polynucleotide (double-stranded or single-stranded) located between the 5' region and the 3' region of a gene as defined herein. The "internal coding region" is not shorter than 8 nucleotides in length and not longer than 1000 nucleotides in length. Other possible lengths of the "internal coding region" include but are not limited to 10, 20, 25, 50, 100, 200, 400, and 500 nucleotides. The 5', 3' and internal regions are non-overlapping and may, but need not be contiguous, and may, but need not, add up to the full length of the corresponding gene.

[0119] As used herein, the term "internal polypeptide region" of a polypeptide refers to the polypeptide sequences located between the amino terminal region and the carboxy terminal region of a polypeptide, as defined herein. The "internal polypeptide region" of a polypeptide is not shorter than 3 amino acids in length and not longer than 350 amino acids in length. Other possible lengths of the "internal polypeptide region" of a polypeptide include, but are not limited to, 5, 10, 20, 25, 50, 100 and 200 amino acids.

[0120] The amino terminal, carboxy terminal and internal polypeptide regions of a polypeptide are non-overlapping and may, but need not be contiguous, and may, but need not, add up to the full length of the corresponding polypeptide.

[0121] As used herein, "isolated" or "purified" when used in reference to a nucleic acid means that a naturally occurring sequence has been removed from its normal cellular (e.g., chromosomal) environment or is synthesized in a non-natural environment (e.g., artificially synthesized). Thus, an "isolated" or "purified" sequence may be in a cell-free solution or placed in a different cellular environment. The term "purified" does not imply that the sequence is the only nucleotide present, but that it is essentially free (about 90-95% pure) of non-nucleotide material naturally associated with it, and thus is distinguished from isolated chromosomes.

[0122] As used herein, the terms "isolated" and "purified" in the context of a proteinaceous agent (e.g., a peptide, polypeptide, protein or antibody) refer to a proteinaceous agent which is substantially free of cellular material and in some embodiments, substantially free of heterologous proteinaceous agents (i.e., contaminating proteins) from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a proteinaceous agent in which the proteinaceous agent is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a proteinaceous agent that is substantially free of cellular material includes preparations of a proteinaceous agent having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous proteinaceous agent (e.g., protein, polypeptide, peptide, or antibody; also referred to as a "contaminating protein"). When the proteinaceous agent is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the proteinaceous agent is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the proteinaceous agent. Accordingly, such preparations of a proteinaceous agent have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the proteinaceous agent of interest. Preferably, proteinaceous agents disclosed herein are isolated.

[0123] As used herein, the term "level of expression" refers to the measurable quantity of a given nucleic acid or protein as determined by methods known to a person skilled in the art and as described herein. In reference to RNA, hnRNA, mRNA or spliced variants of mRNA corresponding to a biomarker of the invention, level of expression can be determined by hybridization or more quantitative measurements such as real-time RT PCR, which includes use of both SYBR® green, TaqMan® and Molecular Beacons technology can be used.

[0124] As used herein, a "ligand" is a molecule that specifically binds to a polypeptide encoded by one of the genes of a biomarker of the invention. A ligand can be a nucleic acid (RNA or DNA), polypeptide, peptide or chemical compound. A ligand of the invention can be a peptide ligand, e.g., a scaffold peptide, a linear peptide, or a cyclic peptide. In a preferred embodiment, the polypeptide ligand is an antibody. The antibody can be a human antibody, a chimeric antibody, a recombinant antibody, a humanized antibody, a monoclonal antibody, or a polyclonal antibody. The antibody can be an intact immunoglobulin, e.g., an IgA, IgG, IgE, IgD, IgM or subtypes thereof. The antibody can be conjugated to a functional moiety (e.g., a compound which has a biological or chemical function (which may be a second different polypeptide, a therapeutic drug, a cytotoxic agent, a detectable moiety, or a solid support. A polypeptide ligand e.g. antibody of the invention interacts with a polypeptide, encoded by one of the genes of a biomarker, with high affinity and specificity. For example, the polypeptide ligand binds to a polypeptide, encoded by one of the genes of a biomarker, with an affinity constant of at least 10^7 M^{-1}, preferably, at least 10^8 M^{-1}, 10^9 M^{-1}, or 10^{10} M^{-1}.

[0125] As used herein, the term "majority" refers to a number representing more than 50% (e.g., 51%, 60%, or 70%, or 80% or 90% or up to 100%) of the total members of a composition. The term "majority", when referring to an array, it means more than 50% (e.g., 51%, 60%, or 70%, or 80% or

90% or up to 100%) of the total nucleic acid members that are stably associated with the solid substrate of the array.

[0126] As used herein, the terms "manage", "managing" and "management" refer to the beneficial effects that a subject derives from a therapy (e.g., a prophylactic or therapeutic agent) which does not result in a cure of osteoarthritis. In certain embodiments, a subject is administered one or more therapies to "manage" osteoarthritis so as to prevent the progression or worsening of the osteoarthritis.

[0127] An "mRNA" means an RNA complimentary to a gene; an mRNA includes a protein coding region and also may include 5' end and 3' untranslated regions (UTR).

[0128] As used herein, "mRNA integrity" refers to the quality of mRNA extracts from either cartilage samples or blood samples. mRNA extracts with good integrity do not appear to be degraded when examined by methods well known in the art, for example, by RNA agarose gel electrophoresis (e.g., Ausubel et al., John Wiley & Sons, Inc., 1997, Current Protocols in Molecular Biology). Preferably, the mRNA samples have good integrity (e.g., less than 10%, preferably less than 5%, and more preferably less than 1% of the mRNA is degraded) to truly represent the gene expression levels of the cartilage or blood samples from which they are extracted.

[0129] As used herein, the terms "non-responsive" and refractory" describe patients treated with a currently available therapy (e.g., prophylactic or therapeutic agent) for osteoarthritis, which is not clinically adequate to relieve one or more symptoms associated therewith. Typically, such patients suffer from severe, persistently active disease and require additional therapy to ameliorate the symptoms associated with their osteoarthritis.

[0130] As used herein, "normal" refers to an individual or group of individuals who have not shown any OA symptoms, including joint pain, and have not been diagnosed with cartilage injury or OA. Preferably said normal individual(s) is not on medication affecting OA and has not been diagnosed with any other disease. More preferably normal individuals have similar sex, age and body mass index (BMI) as compared with the test samples. "Normal", according to the invention, also refers to a samples isolated from normal individuals and includes total RNA or mRNA isolated from normal individuals. A sample taken from a normal individual can include RNA isolated from a cartilage tissue sample wherein RNA is isolated from a whole or a piece of cartilage isolated from cartilage tissue from an individual who was not diagnosed with OA and does not show any symptoms of OA at the time of tissue removal. In one embodiment of the invention, the "normal" cartilage sample is isolated at 14 hours post-mortem and the integrity of mRNA samples extracted is confirmed. A sample taken from a normal individual can also include RNA isolated from a blood sample wherein the blood is from an individual who has not been diagnosed with OA and does not show any symptoms of OA at the time the blood is isolated.

[0131] As used herein, "nucleic acid(s)" is interchangeable with the term "polynucleotide(s)" and it generally refers to any polyribonucleotide or poly-deoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA or any combination thereof. "Nucleic acids" include, without limitation, single- and double-stranded nucleic acids. As used herein, the term "nucleic acid(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acids". The term "nucleic acids" as it is used herein embraces such chemically, enzymatically or metabolically modified forms of nucleic acids, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including for example, simple and complex cells. A "nucleic acid" or "nucleic acid sequence" may also include regions of single- or double-stranded RNA or DNA or any combinations thereof and can include expressed sequence tags (ESTs) according to some embodiments of the invention. An EST is a portion of the expressed sequence of a gene (i.e., the "tag" of a sequence), made by reverse transcribing a region of mRNA so as to make cDNA.

[0132] As defined herein, a "nucleic acid array" refers a plurality of unique nucleic acids (or "nucleic acid members") attached to a support where each of the nucleic acid members is attached to a support in a unique pre-selected region. In one embodiment, the nucleic acid probe attached to the surface of the support is DNA. In a preferred embodiment, the nucleic acid probe attached to the surface of the support is either cDNA or oligonucleotides. In another preferred embodiment, the nucleic acid probe attached to the surface of the support is cDNA synthesized by polymerase chain reaction (PCR). The term "nucleic acid", as used herein, is interchangeable with the term "polynucleotide". In another preferred embodiment, a "nucleic acid array" refers to a plurality of unique nucleic acids attached to nitrocellulose or other membranes used in Southern and/or Northern blotting techniques.

[0133] As used herein, a "nucleic acid probe" or a "nucleic acid marker" or a "nucleic acid member on an array" or "nucleic acid probe on an array" also includes nucleic acid immobilized on an array and capable of binding to a nucleic acid member of complementary sequence through sets of non-covalent bonding interactions, including complementary base pairing interactions. As used herein, a nucleic acid probe may include natural (i. e., A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In addition, the bases in nucleic acid probes may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization (i.e., the nucleic acid probe still specifically binds to its complementary sequence under standard stringent or selective hybridization conditions). Thus, nucleic acid probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages.

[0134] As used herein "nucleic acid target" or "nucleic acid target marker" is defined as a nucleic acid capable of binding to a nucleic acid bound to an array of complementary sequence through sets of non-covalent bonding interactions including complementary base pairing interactions. The nucleic acid target can either be an isolated nucleic acid sequence corresponding to a gene or portion thereof, or the nucleic acid target can be total RNA or mRNA isolated from a sample. Preferably, the nucleic acid target or nucleic acid markers are derived from human cartilage, blood, or synovial fluid extracts. More preferably, the nucleic acid targets are single- or double-stranded DNA, RNA, or DNA-RNA hybrids, from human cartilage, blood, or synovial fluid total RNA extracts, and preferably from mRNA extracts.

[0135] In one embodiment, a conventional nucleic acid array of 'target' sequences bound to the array can be representative of the entire human genome, e.g. Affymetrix chip, and the isolated biomarker consisting of or comprising two or more of the 19 genes described in FIG. **1** or gene probes is applied to the conventional array.

[0136] In another embodiment, sequences bound to the array can be an isolated oligonucleotide, cDNA, EST or PCR product corresponding to a biomarker of the invention total cellular RNA is applied to the array.

[0137] As used herein, the term "oligonucleotide" is defined as a molecule comprised of two or more deoxyribonucleotides and/or ribonucleotides, and preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide. The oligonucleotides may be from about 8 to about 1,000 nucleotides long. Although oligonucleotides of 8 to 100 nucleotides are useful in the invention, preferred oligonucleotides range from about 8 to about 15 bases in length, from about 8 to about 20 bases in length, from about 8 to about 25 bases in length, from about 8 to about 30 bases in length, from about 8 to about 40 bases in length or from about 8 to about 50 bases in length.

[0138] As used herein, "osteoarthritis" refers to a particular form of arthritis, and in particular a chronic disease in which the articular cartilage that lies on the ends of bones that form the articulating surface of the joints gradually degenerates over time. Cartilage degeneration can be caused by an imbalanced catabolic activity (removal of "old" cells and matrix components) and anabolic activity (production of "new" cells and molecules) (Westacott et al., 1996, Semin Arthritis Rheum. 25:254-72).

[0139] As used herein, the term "osteoarthritis (OA) staging" or "osteoarthritis (OA) grading" refers to determining the presence, the absence, and or the degree of advancement or progression of OA in an individual. "OA stages" or "OA grades" include "mild OA", "moderate OA", "marked OA" and "severe OA" and "no OA" as defined in accordance with the scoring system of Marshall described below, but are not limited to these stages. For example one could use an even more refined scoring system of that taught by Marshall wherein smaller categories are defined (e.g. "mild OA" can be subdivided into categories as can moderate, marked and severe on the basis of the Marshall score) In one embodiment, one can utilize traditional techniques to classify the stages of OA. Many scoring system are known in the art, and can be used, for example radiography with a Kellgren-Lawrence score as an alternative means of OA diagnosis and staging. It would be understood by a person skilled in the art how to interpret "mild OA", "moderate OA" "marked OA" and "severe OA" in accordance with other classification systems. In order to classify cartilage into different disease stages, preferably the scoring system described in Marshall (Marshall W., 1996, The Journal of Rheumatology, 23:582-584, incorporated by reference) is used. According to this method, each of the 6 articular surfaces (patella, femoral trochlea, medial femoral condyle, medial tibial plateau, lateral femoral condyle and lateral tibial plateau) is assigned a cartilage grade based on the worst lesion present on that specific surface. A scoring system is then applied in which each articular surface receives an OA severity number value that reflects the cartilage severity grade for that surface. For example, if the medial femoral condyle has a grade I lesion as its most severe cartilage damage a value of 1 is assigned. A total score for the patient is then derived from the sum of the scores on the 6 articular surfaces. Based on the total score, each patient is placed into one of 4 OA groups: "mild" (early) is defined as having a Marshall score of 1-6, "moderate" is defined as having a Marshall score of 7-12, "marked" is defined as having a Marshall score of 13-18 and "severe" is defined as having a Marshall score of greater than 18. In another embodiment, one can measure the expression of the biomarkers of the invention in accordance with the teachings herein in order to determine the presence and or the degree of advancement or progression of the disease.

[0140] As used herein, the phrase "pharmaceutically acceptable salt(s)," includes, but is not limited to, salts of acidic or basic groups that may be present in compounds identified using the methods of the present invention. Compounds that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that can be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, including but not limited to sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds that are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium lithium, zinc, potassium, and iron salts.

[0141] As used herein, "polynucleotide" encompasses double-stranded DNA, single-stranded DNA and double-stranded or single-stranded RNA of more than 8 nucleotides in length.

[0142] As used herein, "polypeptide sequences encoded by" refers to the amino acid sequences obtained after translation of the protein coding region of a gene, as defined herein. The mRNA nucleotide sequence for each of the 19 genes is identified by its Genbank Accession number (see FIGS. **1-5**) and the corresponding polypeptide sequence is identified by a Protein Accession number (see FIGS. **1-5**). The Genbank Accession numbers identified in FIGS. **1-5** provide the location of the 5' UTR, protein coding region (CDS) and 3' UTR within the mRNA nucleotide sequence of each of the 19 genes.

[0143] When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as epitopes or antigenic determinants. As used herein, "antigenic fragments" refers portions of a polypeptide that contains one or more epitopes. Epitopes can be linear, comprising essentially a linear sequence from the antigen, or conformational, comprising sequences which are genetically separated by other sequences but come together structurally at the binding site for the polypeptide ligand. "Antigenic fragments"may be 5000, 1000, 500, 400, 300, 200, 100, 50 or 25 or 20 or 10 or 5 amino acids in length.

[0144] As used herein, "pre-selected region", "predefined region", or "unique position" refers to a localized area on a substrate which is, was, or is intended to be used for the deposit of a nucleic acid and is otherwise referred to herein in

the alternative as a "selected region" or simply a "region." The pre-selected region may have any convenient shape, e.g., circular, rectangular, elliptical, wedge-shaped, etc. In some embodiments, a pre-selected region is smaller than about 1 cm^2, more preferably less than 1 mm^2, still more preferably less than 0.5 mm^2, and in some embodiments less than 0.1 mm^2. A nucleic acid member at a "pre-selected region", "predefined region", or "unique position" is one whose identity (e.g., sequence) can be determined by virtue of its position at the region or unique position.

[0145] As used herein, the terms "prevent", "preventing" and "prevention" refer to the prevention of the development, recurrence or onset of osteoarthritis or one or more symptoms thereof resulting from the administration of one or more compounds identified in accordance the methods of the invention or the administration of a combination of such a compound and another therapy.

[0146] The term, "primer", as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and the method used. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. The factors involved in determining the appropriate length of primer are readily known to one of ordinary skill in the art.

[0147] As used herein, the term "probe" means oligonucleotides and analogs thereof and refers to a range of chemical species that recognize polynucleotide target sequences through hydrogen bonding interactions with the nucleotide bases of the target sequences. The probe or the target sequences may be single- or double-stranded RNA or single- or double-stranded DNA or a combination of DNA and RNA bases. A probe is at least 8 nucleotides in length and less than the length of a complete gene. A probe may be 10, 20, 30, 50, 75, 100, 150, 200, 250, 400, 500 and up to 2000 nucleotides in length as long as it is less the full length of the target gene. Probes can include oligonucleotides modified so as to have a tag which is detectable by fluorescence, chemiluminescence and the like. The probe can also be modified so as to have both a detectable tag and a quencher molecule, for example Taqman® and Molecular Beacon® probes.

[0148] The oligonucleotides and analogs thereof may be RNA or DNA, or analogs of RNA or DNA, commonly referred to as antisense oligomers or antisense oligonucleotides. Such RNA or DNA analogs comprise but are not limited to 2-O-alkyl sugar modifications, methylphosphonate, phosphorothiate, phosphorodithioate, formacetal, 3'-thioformacetal, sulfone, sulfamate, and nitroxide backbone modifications, and analogs wherein the base moieties have been modified. In addition, analogs of oligomers may be polymers in which the sugar moiety has been modified or replaced by another suitable moiety, resulting in polymers which include, but are not limited to, morpholino analogs and peptide nucleic acid (PNA) analogs (Egholm, et al. Peptide Nucleic Acids (PNA)—Oligonucleotide Analogues with an Achiral Peptide Backbone, (1992)).

[0149] Probes may also be mixtures of any of the oligonucleotide analog types together or in combination with native DNA or RNA. At the same time, the oligonucleotides and analogs thereof may be used alone or in combination with one or more additional oligonucleotides or analogs thereof.

[0150] As used herein, the terms "prophylactic agent" and "prophylactic agents" refer to any compound(s) which can be used in the prevention of osteoarthritis. In certain embodiments, the term "prophylactic agent" refers to a compound identified in the screening assays described herein. In certain other embodiments, the term "prophylactic agent" refers to an agent other than a compound identified in the screening assays described herein which is known to be useful for, or has been or is currently being used to prevent or impede the onset, development and/or progression of osteoarthritis or one or more symptoms thereof.

[0151] As used herein, the phrase "prophylactically effective amount" refers to the amount of a therapy (e.g., a prophylactic agent) which is sufficient to result in the prevention of the development, recurrence or onset of osteoarthritis or one or more symptoms thereof.

[0152] As used herein, the terms "protein" and "polypeptide" are used interchangeably to refer to a chain of amino acids linked together by peptide bonds. In a specific embodiment, a protein is composed of less than 200, less than 175, less than 150, less than 125, less than 100, less than 50, less than 45, less than 40, less than 35, less than 30, less than 25, less than 20, less than 15, less than 10, or less than 5 amino acids linked together by peptide bonds. In another embodiment, a protein is composed of at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500 or more amino acids linked together by peptide bonds.

[0153] A "protein coding region" refers to the portion of the mRNA encoding a polypeptide.

[0154] As used herein the term "polynucleotide which selectively hybridizes to a product of the Biomarker of the invention" refers to

[0155] As used herein, "a plurality of" or "a set of" refers to more than two, for example, 3 or more, 10 or more, 100 or more, or 1000 or more, or 10,000 or more.

[0156] As used herein, the terms "a portion thereof" and "RNA portion" in context of RNA products of a biomarker of the invention refer to an RNA transcript comprising a nucleic acid sequence of at least 6, at least 9, at least 15, at least 18, at least 21, at least 24, at least 30, at least 60, at least 90, at least 99, or at least 108, or more nucleotides of a RNA product of a biomarker of the invention.

[0157] As used herein the term "product of the biomarker of the invention" refers to the RNA and/or the protein expressed by the gene corresponding to the biomarker of the invention. The "RNA product of a biomarker of the invention" includes one or more products which can include heteronuclear RNA ("hnRNA"), mRNA, and all or some of the spliced variants of mRNA whose measure of expression can be used as a biomarker in accordance with the teachings disclosed herein. The "protein product of a biomarker of the invention" includes one or more of the products of the biomarker which can include proteins, protein variants, and any post-translationally modified proteins.

[0158] As used herein, the term "selectively binds" in the context of the invention refers to the interaction of a any two

of a peptide, a protein, a polypeptide an antibody, wherein the interaction is dependent upon the presence of particular structures on the respective molecules. For example, when the two molecules are protein molecules, a structure on the first molecule recognizes and binds to a structure on the second molecule, rather than to other proteins. "Selective binding", as the term is used herein, means that a molecule binds to its specific binding partner with a specificity of at least 70%, at least 80%, at least 90% and most preferably 100% specificity.

[0159] As used herein "selective hybridization" in the context of this invention refers to a hybridization which occurs as between a polynucleotide encompassed by the invention and an RNA or protein product of the biomarker of the invention wherein the hybridization is such that the polynucleotide is specific for the RNA or protein product or products of the biomarker of the invention to the exclusion of RNA or protein products of other genes in the genome in question. In a preferred embodiment a polynucleotide which "selectively hybridizes" is one which hybridizes with a specificity of greater than 70%, greater than 80%, greater than 90% and most preferably 100% specificity. As would be understood to a person skilled in the art, a polynucleotide which "selectively hybridizes" to the RNA product of a biomarker of the invention can be determined taking into account the length and composition.

[0160] As used herein, the term "significant match", when referring to nucleic acid sequences, means that two nucleic acid sequences exhibit at least 65% identity, at least 70%, at least 75%, at least 80%, at least 85%, and preferably, at least 90% identity, using comparison methods well known in the art (i.e., Altschul, S.F. et al., 1997, Nucl. Acids Res., 25:3389-3402; Schaffer, A.A. et al., 1999, Bioinformatics 15:1000-1011). As used herein, "significant match" encompasses non-contiguous or scattered identical nucleotides so long as the sequences exhibit at least 65%, and preferably, at least 70%, at least 75%, at least 80%, at least 85%, and preferably, at least 90% identity, when maximally aligned using alignment methods routine in the art.

[0161] As used herein, the terms "solid substrate" and "solid support" refers to a material having a rigid or semi-rigid surface. The terms "substrate" and "support" are used interchangeably herein with the terms "solid substrate" and "solid support". The solid support may be biological, non-biological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, beads, containers, capillaries, pads, slices, films, plates, slides, chips, etc. Often, the substrate is a silicon or glass surface, (poly)tetrafluoroethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, a charged membrane, such as nylon 66 or nitrocellulose, or combinations thereof. In a preferred embodiment, the solid support is glass. Preferably, at least one surface of the substrate will be substantially flat. Preferably, the solid support will contain reactive groups, including, but not limited to, carboxyl, amino, hydroxyl, thiol, and the like. In one embodiment, the solid support is optically transparent.

[0162] Solid supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, polystyrene beads, alumina gels, and polysaccharides. A suitable solid support may be selected on the basis of desired end use and suitability for various synthetic protocols. For example, for peptide synthesis, a solid support can be a resin such as p-methylbenzhydrylamine (pMBHA) resin (Peptides International, Louisville, Ky.), polystyrenes (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), including chloromethylpolystyrene, hydroxymethylpolystyrene and aminomethylpolystyrene, poly(dimethylacrylamide)-grafted styrene co-divinyl-benzene (e.g., POLYHIPE resin, obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (e.g., TENTAGEL or ARGOGEL, Bayer, Tubingen, Germany) polydimethylacrylamide resin (obtained from Milligen/Biosearch, California), or Sepharose (Pharmacia, Sweden).

[0163] As used herein, the term "specifically binds" refers to the interaction of two molecules, e.g., a ligand and a protein or peptide, or an antibody and a protein or peptide wherein the interaction is dependent upon the presence of particular structures on the respective molecules. For example, when the two molecules are protein molecules, a structure on the first molecule recognizes and binds to a structure on the second molecule, rather than to proteins in general. "Specific binding", as the term is used herein, means that a molecule binds its specific binding partner with at least 2-fold greater affinity, and preferably at least 10-fold, 20-fold, 50-fold, 100-fold or higher affinity than it binds a non-specific molecule.

[0164] As used herein, "specifically hybridizes", "specific hybridization" refers to hybridization which occurs when two nucleic acid sequences are substantially complementary (at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, preferably at least about 75% complementary, more preferably at least about 90% complementary). See Kanehisa, M., 1984, Nucleic acids Res., 12:203, incorporated herein by reference. As a result, it is expected that a certain degree of mismatch is tolerated. Such mismatch may be small, such as a mono-, di- or tri-nucleotide. Alternatively, a region of mismatch can encompass loops, which are defined as regions in which there exists a mismatch in an uninterrupted series of four or more nucleotides. Numerous factors influence the efficiency and selectivity of hybridization of two nucleic acids, for example, the hybridization of a nucleic acid member on an array to a target nucleic acid sequence. These factors include nucleic acid member length, nucleotide sequence and/or composition, hybridization temperature, buffer composition and potential for steric hindrance in the region to which the nucleic acid member is required to hybridize. A positive correlation exists between the nucleic acid length and both the efficiency and accuracy with which a nucleic acid will anneal to a target sequence. In particular, longer sequences have a higher melting temperature (T_M) than do shorter ones, and are less likely to be repeated within a given target sequence, thereby minimizing promiscuous hybridization. Hybridization temperature varies inversely with nucleic acid member annealing efficiency. Similarly the concentration of organic solvents, e.g., formamide, in a hybridization mixture varies inversely with annealing efficiency, while increases in salt concentration in the hybridization mixture facilitate annealing. Under stringent annealing conditions, longer nucleic acids, hybridize more efficiently than do shorter ones, which are sufficient under more permissive conditions.

[0165] As used herein, "stably associated" refers to a nucleic acid that is stably bound to a solid substrate to form an array via covalent bonds, hydrogen bonds or ionic interactions such that the nucleic acid retains its unique pre-selected position relative to all other nucleic acids that are stably associated with an array, or to all other pre-selected regions on the solid substrate under conditions in which an array is

typically analyzed (i.e., during one or more steps of hybridization, washes, and/or scanning, etc.).

[0166] As herein used, the term "standard stringent conditions" means hybridization will occur only if there is at least 95% and preferably, at least 97% identity between the sequences, wherein the region of identity comprises at least 10 nucleotides. In one embodiment, the sequences hybridize under stringent conditions following incubation of the sequences overnight at 42° C., followed by stringent washes (0.2×SSC at 65° C.). The degree of stringency of washing can be varied by changing the temperature, pH, ionic strength, divalent cation concentration, volume and duration of the washing. For example, the stringency of hybridization may be varied by conducting the hybridization at varying temperatures below the melting temperatures of the probes. The melting temperature of the probe may be calculated using the following formulas:

[0167] For oligonucleotide probes, between 14 and 70 nucleotides in length, the melting temperature (Tm) in degrees Celsius may be calculated using the formula: Tm=81.5+16.6(log [Na+])+0.41(fraction G+C)−(600/N) where N is the length of the oligonucleotide.

[0168] For example, the hybridization temperature may be decreased in increments of 5° C. from 68° C. to 42° C. in a hybridization buffer having a Na+ concentration of approximately 1M. Following hybridization, the filter may be washed with 2×SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate stringency" conditions above 50° C. and "low stringency" conditions below 50° C. A specific example of "moderate stringency" hybridization conditions is when the above hybridization is conducted at 55° C. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 45° C.

[0169] If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation Tm=81.5+16.6(log [Na^+])+0.41(fraction G+C)−(0.63% formamide)−(600/N), where N is the length of the probe.

[0170] For example, the hybridization may be carried out in buffers, such as 6×SSC, containing formamide at a temperature of 42° C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6×SSC, 0.5% SDS at 50° C. These conditions are considered to be "moderate stringency" conditions above 25% formamide and "low stringency" conditions below 25% formamide. A specific example of "moderate stringency" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

[0171] As used herein, the terms "subject" and "patient" and "individual" are used interchangeably to refer to an animal (e.g., a mammal, a fish, an amphibian, a reptile, a bird and an insect). In a specific embodiment, a subject is a mammal (e.g., a non-human mammal and a human). In another embodiment, a subject is a pet (e.g., a dog, a cat, a guinea pig, a monkey and a bird), a farm animal (e.g., a horse, a cow, a pig, a goat and a chicken) or a laboratory animal (e.g., a mouse and a rat). In another embodiment, a subject is a primate (e.g., a chimpanzee and a human). In a preferred embodiment, a subject is a human.

[0172] As used herein, the term "synergistic" refers to a combination of a compound identified using one of the methods described herein, and another therapy (e.g., agent), which is more effective than the additive effects of the therapies. Preferably, such other therapy has been or is currently being to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. A synergistic effect of a combination of therapies (e.g., prophylactic or therapeutic agents) permits the use of lower dosages of one or more of the therapies and/or less frequent administration of said therapies to a subject with osteoarthritis. The ability to utilize lower dosages of a therapy (e.g., a prophylactic or therapeutic agent) and/or to administer said therapy less frequently reduces the toxicity associated with the administration of said agent to a subject without reducing the efficacy of said therapies in the prevention, treatment, management or amelioration of osteoarthritis. In addition, a synergistic effect can result in improved efficacy of therapies (e.g., agents) in the prevention, treatment, management or amelioration of osteoarthritis. Finally, a synergistic effect of a combination of therapies (e.g., prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of either therapy alone.

[0173] As used herein, "synovial fluid" refers to fluid secreted from the "synovial sac" which surrounds each joint. Synovial fluid serves to protect the joint, lubricate the joint and provide nourishment to the articular cartilage. Synovial fluid useful according to the invention contains cells from which RNA can be isolated according to methods well known in the art as described herein.

[0174] As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to any compound(s) which can be used in the treatment, management or amelioration of osteoarthritis or one or more symptoms thereof. In a specific embodiment, the term "therapeutic agent" refers to a compound that increases or decreases the expression of a polynucleotide sequence that is differentially expressed in a chondrocyte from any two of the following developmental or osteoarthritis disease stages: (a) mild, (b) moderate, (c) marked and (d) severe, or (e) chondrocyte from a normal individual, as defined herein. A therapeutic agent according to the invention also refers to a compound that increases or decreases the anabolic activity of a chondrocyte. The invention provides for a "therapeutic agent" that 1) prevents the onset of osteoarthritis; 2) reduces, delays, or eliminates osteoarthritis symptoms such as pain, swelling, weakness and loss of functional ability in the afflicted joints; 3) reduces, delays, or eliminates cartilage degeneration, and/or enhances chondrocyte metabolic activity and cell division rates; and/or 4) restores one or more expression profiles of one or more disease-indicative nucleic acids of a patient to a profile more similar to that of a normal individual when administered to a patient. In certain embodiments, the term "therapeutic agent" refers to a compound identified in the screening assays described herein. In other embodiments, the term "therapeutic agent" refers to an agent other than a compound identified in the screening assays described herein which is known to be useful for or has been or is currently being used to treat, manage or ameliorate osteoarthritis or one or more symptoms thereof.

[0175] As used herein, the term "therapeutically effective amount" refers to that amount of a therapy (e.g., a therapeutic

agent) sufficient to result in the amelioration of osteoarthritis or one or more symptoms thereof, prevent advancement of osteoarthritis, cause regression of osteoarthritis, or to enhance or improve the therapeutic effect(s) of another therapy (e.g., therapeutic agent). In a specific embodiment, a therapeutically effective amount refers to the amount of a therapy (e.g., a therapeutic agent) that reduces joint pain or swelling of the joint. Preferably, a therapeutically effective of a therapy (e.g., a therapeutic agent) reduces the swelling of the joint by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% relative to a control such as phosphate buffered saline ("PBS").

[0176] As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of osteoarthritis or one or more symptoms thereof resulting from the administration of one or more compounds identified in accordance the methods of the invention, or a combination of one or more compounds identified in accordance with the invention and another therapy.

[0177] As used herein, the term "up regulated" or "increased level of expression" in the context of this invention refers to a sequence corresponding to a gene which is expressed wherein the measure of the quantity of the sequence demonstrates an increased level of expression of the gene, as can be determined using array analysis or other similar analysis, in cartilage or blood isolated from an individual having osteoarthritis or an identified disease state of osteoarthritis as determined by osteoarthritis staging as compared with the same gene in cartilage or blood isolated from normal individuals or from an individual with a different identified disease state of osteoarthritis as determined by osteoarthritis staging. An "increased level of expression" according to the present invention, is an increase in expression of at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% or more, or greater than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 50-fold, 100-fold or more as measured, for example, by the intensity of hybridization according to methods of the present invention. For example, up regulated sequences includes sequences having an increased level of expression in cartilage or blood isolated from individuals characterized as having mild, moderate, marked or severe OA as compared with cartilage isolated from normal individuals. Up regulated sequences can also include sequences having an increased level of expression in cartilage or blood isolated from individuals characterized as having one stage of osteoarthritis as compared to another stage of osteoarthritis (e.g. marked OA v. severe OA).

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0178] The objects and features of the invention can be better understood with reference to the following detailed description and drawings.

[0179] FIG. **1** is a figure showing the polynucleotide and polypeptide sequences of 19 genes that are differentially regulated in a population of mild OA individuals when compared with the same genes in a population of normal individuals.

[0180] FIG. **2** is a figure showing the polynucleotide and polypeptide sequences of 4 genes that are differentially regulated in a population of individuals with moderate OA as compared with a population of normal individuals.

[0181] FIG. **3** is a figure showing the polynucleotide and polypeptide sequences of 2 genes that are differentially regulated in a population of OA individuals with moderate OA as compared with a population of individuals having marked OA.

[0182] FIG. **4** is a figure showing the polynucleotide and polypeptide sequences of 4 genes that are differentially regulated in a population of individuals with marked OA as compared with a population of individuals with severe OA.

[0183] FIG. **5** shows the differential Alpha glucosidase II alpha subunit (G2AN) gene expression in blood samples from patients with mild vs moderate OA using QRT-PCR analysis.

[0184] FIG. **6** shows the differential Tumor necrosis factor, alpha-induced protein 6 (TNFAIP6) gene expression in blood samples from patients with mild vs moderate OA using SYBR® Green QRT-PCR and TaqMan® analysis.

[0185] FIG. **7** shows the differential Period homolog 1 (*Drosophila*) (PER1) gene expression in blood samples from patients with marked vs severe OA using QRT-PCR analysis.

[0186] FIG. **8** shows the differential Zinc finger RNA binding protein (ZFR) gene expression in blood samples from patients with moderate vs marked OA using Taqman® QRT-PCR analysis.

[0187] FIG. **9** is a figure showing differential ATP-binding cassette, sub-family A, member 1(ABCA1) and ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1) gene expression in blood samples from patients with mild vs marked OA using QRT-PCR analysis.

[0188] FIG. **10** is a figure showing differential Interferon Regulatory Factor 1 (IRF1) gene expression in blood samples from patients with mild vs marked OA using QRT-PCR analysis.

[0189] FIG. **11** is a figure showing differential Nuclear Receptor Co-Activator 1 (NRCOA1) and Chloride Intracellular Channel 4 (CLIC4) gene expression in blood samples from patients with mild vs marked OA using QRT-PCR analysis.

[0190] Table 1 is a table showing, in one embodiment, the genes of the invention and in particular identifying the genes on the basis of their locus link ID.

[0191] Table 2 is a table showing, specific embodiments of the genes of the invention and in the reference accession numbers corresponding mRNA transcripts and proteins corresponding to the genes of the invention.

5. DETAILED DESCRIPTION OF THE INVENTION

[0192] The practice of the present invention employs in part conventional techniques of molecular biology, microbiology and recombinant DNA techniques, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, 1989, *Molecular Cloning: A Laboratory Manual,* Second Edition; *Oligonucleotide Synthesis* (M. J. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. D. Harnes & S. J. Higgins, eds., 1984); *A Practical Guide to Molecular Cloning* (B. Perbal, 1984); and a series, *Methods in Enzymology* (Academic Press, Inc.); *Short Protocols In Molecular Biology,* (Ausubel et al., ed., 1995). All patents, patent applications, and publications mentioned herein, both supra and infra, are hereby incorporated by reference in their entireties.

[0193] The invention as disclosed herein identifies biomarkers and biomarker combinations useful in diagnosing OA and/or in diagnosing stages of OA. In order to use these biomarkers, the invention teaches the identification of the products of these biomarkers including the RNA products and the protein products. The invention further discloses the oligonucleotides, cDNA, DNA, RNA, PCR products, synthetic DNA, synthetic RNA, or other combinations of naturally occurring of modified nucleotides that specifically and/or selectively hybridize to the RNA products of the biomarkers of the invention. The invention further discloses proteins, peptides, antibodies, ligands that specifically and/or selectively hybridize to the protein products of the biomarkers of the invention. The measuring of the expression of the RNA product of the biomarkers and combination of biomarkers of the invention, can be done by using those polynucleotides which are specific and/or selective for the RNA products of the biomarkers of the invention to quantitate the expression of the RNA product. In a specific embodiment of the invention, the polynucleotides which are specific and/or selective for the RNA products are probes or primers. In one embodiment, these polynucleotides are in the form of a nucleic acid probe which can be hybridized to a manufactured array. In another embodiment, commercial arrays can be used to measure the expression of the RNA product and the invention teaches which combination of genes to analyze. In another embodiment, the polynucleotides which are specific and/or selective for the RNA products of the biomarkers of the invention are used in the form of probes and primers in techniques such as quantitative real-time RT PCR, using for example SYBR®Green, or using TaqMan® or Molecular Beacon techniques, where the polynucleotides used are used in the form of a forward primer, a reverse primer, a TaqMan labelled probe or a Molecular Beacon labelled probe. In one specific embodiment, the results generated from measuring the level of expression of the RNA products of the invention can be input into the model of the invention which is used to identify the combinations of biomarkers to determine a diagnosis as defined by the model. In a preferred embodiment, the same method is used to generate the expression data used to generate the mathematical model as is used to diagnose the test individual.

[0194] The invention further contemplates the use of proteins or polypeptides as disclosed herein and would be known by a person skilled in the art to measure the protein products of the biomarkers of the invention. Techniques known to persons skilled in the art (for example, techniques such as Western Blotting, Immunoprecipitation protein microarray analysis and the like) can then be used to measure the level of protein products corresponding to the biomarkers of the invention. As would be understood to a person skilled in the art, the measure of the level of expression of the protein products of the biomarkers of the invention requires a protein which specifically or selectively binds to one or more of the protein products corresponding to each biomarker of the invention. Data representative of the level of expression of the protein products of the biomarker of the invention can then be input into the model generated to identify the combination in order to determine a diagnosis as defined by the model. In a preferred embodiment, the same method is used to generate the expression data used to generate the mathematical model as is used to diagnose the test individual.

5.1 Samples for Use in the Invention

[0195] Unless otherwise indicated herein, any tissue sample (e.g., a cartilage, synovial fluid or blood sample) or cell sample (e.g., chondrocyte sample or a blood cell sample) obtained from any subject may be used in accordance with the methods of the invention. Examples of subjects from which such a sample may be obtained and utilized in accordance with the methods of the invention include, but are not limited to, asymptomatic subjects, subjects manifesting or exhibiting 1, 2, 3, 4 or more symptoms of osteoarthritis, subjects clinically diagnosed as having osteoarthritis, subjects predisposed to osteoarthritis (e.g., subjects with a family history of osteoarthritis, subjects with a genetic predisposition to osteoarthritis, and subjects that lead a lifestyle that predisposes them to osteoarthritis or increases the likelihood of contracting osteoarthritis), subjects suspected of having osteoarthritis, subjects undergoing therapy for osteoarthritis, subjects with osteoarthritis and at least one other condition (e.g., subjects with 2, 3, 4, 5 or more conditions), subjects not undergoing therapy for osteoarthritis, subjects determined by a medical practitioner (e.g., a physician) to be healthy or osteoarthritis-free (i.e., normal), subjects that have been cured of osteoarthritis, subjects that are managing their osteoarthritis, and subjects that have not been diagnosed with osteoarthritis. In a specific embodiment, the subjects from which a sample may be obtained and utilized have osteoarthritis of the hands, feet, spine, knee, hip and/or wrist.

[0196] In another embodiment, the subjects from which a sample may be obtained and utilized have mild, marked, moderate or severe osteoarthritis. In a further embodiment, the subject from which a sample may be obtained is a test individual wherein it is unknown whether the person has osteoarthritis, and/or it is unknown what stage of osteoarthritis the test individual has.

[0197] In order to classify an individual according to disease state, a scoring system based on cartilage may be used, whereby subjective decisions by the arthroscopist are minimized. An example of a scoring system which defines disease states described herein is that of Marshall, 1996, The Journal of Rheumatology 23:582-584, incorporated herein by reference. According to this method, each of the 6 articular surfaces (patella, femoral trochlea, medial femoral condyle, medial tibial plateau, lateral femoral condyle and lateral tibial plateau) is assigned a cartilage grade based on the worst lesion present on that specific surface. A scoring system is then applied in which each articular surface receives an osteoarthritis severity number value that reflects the cartilage severity grade for that surface, as described in Table 3.

TABLE 3

Articular Cartilage Grading System

Grade	Articular Cartilage	Points
0	Normal	0
I	Surface intact-softening, edema	1
II	Surface-disrupted-partial thickness lesions (no extension to bone)	2
III	Full thickness lesions-extensions to intact bone	3
IV	Bone erosion or eburnation	4

[0198] For example, if the medial femoral condyle has a grade I lesion as its most severe cartilage damage, a value of 1 is assigned. A total score for the patient is then derived from the sum of the scores of the 6 articular surfaces. Based on the total score, each patient is placed into one of 4 osteoarthritis groups: mild (1-6), moderate (7-12), marked (13-18) and severe (>18).

[0199] In certain embodiments, the sample obtained from a subject is a cartilage sample (including a sample of cells from cartilage). In other embodiments, the sample obtained from a subject is a synovial fluid sample (including a sample of cells from synovial fluid). In yet other embodiments, the sample obtained from a subject is a blood sample (including a sample of cells from blood).

5.1.1 Cartilage

[0200] In one aspect, a cartilage sample is obtained from a fetus using methods known in the art. The chondrocytes of fetal cartilage have a higher level of metabolic activity and cell division rates as compared to chondrocytes from cartilage from either a normal adult or from an individual diagnosed with any stage of osteoarthritis (mild, moderate, marked and severe).

[0201] In another aspect, a cartilage sample is obtained from a normal individual who is alive or is obtained from cartilage tissue less than 14 hours post mortem, according to methods known in the art and described below. Normal articular cartilage from human adults are obtained using any known method. In a specific embodiment, cartilage is obtained from individuals undergoing arthroscopy or total knee replacements and samples are stored in liquid nitrogen until needed. Typically, truly normal cartilage cannot generally be sampled from live donors due to ethical considerations. Thus, preferably, normal cartilage samples are obtained from deceased donors, within a fourteen-hour post-mortem window after cessation of perfusion to the sampled joint, to minimize the degradation of RNA observed beyond the window. In other embodiments, the "normal" tissue is obtained less than 14 hours post-mortem, such as 13, 12, 11, 10, 9, 8, 6, 4, 2, or 1 hour post-mortem. Preferably, the normal cartilage is obtained less than 12 hours post-mortem.

[0202] In another aspect, cartilage is obtained from a subject diagnosed with one of the following disease stages of osteoarthritis: mild, marked, moderate or severe. Human cartilage samples from osteoarthritic individuals are obtained using any known method. Preferably, the cartilage is obtained from individuals undergoing arthroscopy or total knee replacements and samples are stored in liquid nitrogen until needed. In a specific embodiment, a minimum of 0.05 g of cartilage sample is isolated to obtain 2 μg total RNA extract for the construction of a cDNA library. In another embodiment, a minimum of 0.025 g cartilage sample is isolated to obtain 1 μg total RNA extract to use as a target sample for a microarray. A cartilage sample that is useful according to the invention is in an amount that is sufficient for the detection of one or more nucleic acid sequences or amino acid sequences according to the invention.

[0203] The cartilage collected is optionally but preferably stored at refrigerated temperatures, such 4° C., prior to use in accordance with the methods of the invention. In some embodiments, a portion of the cartilage sample is used in accordance with the methods of the invention at a first instance of time whereas one or more remaining portions of the sample is stored for a period of time for later use. This period of time can be an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely. For long term storage, storage methods well known in the art, such as storage at cryo temperatures (e.g., below −60° C.) can be used. In some embodiments, in addition to storage of the cartilage or instead of storage of the cartilage, isolated nucleic acid or protein are stored for a period of time (e.g., an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely) for later use.

[0204] In some embodiments of the present invention, chondrocytes present in the cartilage are separated using techniques known in the art and used in accordance with the methods of the invention. Chondrocytes may be obtained from a subject having any of the following developmental or disease stages: fetal, normal, mild, osteoarthritic, moderate osteoarthritic, marked osteoarthritic or severe osteoarthritic. Chondrocytes can be frozen by standard techniques prior to use in the present methods.

5.1.2 Synovial Fluid

[0205] In one aspect, a sample of synovial fluid is obtained from a subject according to methods well known in the art. For example, arthrocentesis may be performed. During arthrocentesis, a sterile needle is used to remove synovial fluid from a joint. Synovial fluid may be collected from a knee, elbow, wrist, finger, hip, spine or any other joint using arthrocentesis. In a specific embodiment, synovial fluid is collected from the joint affected or suspected to be affected by osteoarthritis. Synovial fluid may be obtained from a subject having any of the following developmental or disease stages: fetal, normal, mild, osteoarthritic, moderate osteoarthritic, marked osteoarthritic or severe osteoarthritic.

[0206] A synovial fluid sample that is useful according to the invention is in an amount that is sufficient for the detection of one or more nucleic acid or amino acid sequences according to the invention. In a specific embodiment, a synovial fluid sample useful according to the invention is in an amount ranging from 0.1 ml to 20 ml, 0.1 ml to 15 ml, 0.1 ml to 10 ml, 0.1 ml to 5 ml, 0.1 to 2 ml, 0.5 ml to 20 ml, 0.5 ml to 15 ml, 0.5 ml to 10 ml, 0.5 ml to 5 ml, or 0.5 ml to 2 ml. In another embodiment, a synovial fluid sample useful according to the invention is 0.1 ml or more, 0.5 ml or more, 1 ml or more, 2 ml or more, 3 ml or more, 4 ml or more, 5 ml or more, 6 ml or more, 7 ml or more, 8 ml or more, 9 ml or more, 10 ml or more, 11 ml or more, 12 ml or more, 13 ml or more, 14 ml or more, 15 ml or more, 16 ml or more, 17 ml or more, 18 ml or more, 19 ml or more, or 20 ml or more.

[0207] The synovial fluid collected is optionally but preferably stored at refrigerated temperatures, such 4° C., prior to use in accordance with the methods of the invention. In some embodiments, a portion of the synovial fluid sample is used in accordance with the methods of the invention at a first instance of time whereas one or more remaining portions of the sample is stored for a period of time for later use. This period of time can be an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely. For long term storage, storage methods well known in the art, such as storage at cryo temperatures (e.g., below −60° C.) can be used. In some embodiments, in addition to storage of the synovial fluid or instead of storage of the synovial fluid, isolated nucleic acid or protein are stored for a period of time (e.g., an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely) for later use.

[0208] In some embodiments of the present invention, cells present in the synovial fluid are separated using techniques known in the art and used in accordance with the methods of the invention. Generally, the following cells are found in synovial fluid: lymphocytes (B and T lymphocytes), monocytes, neutrophils, synoviocytes and macrophages. In synovial fluid from patients with a pathological condition, such as osteoarthritis, the following cells may also be found: chon-

drocytes, osteoblasts and osteoclasts. Such cells may be isolated and used in accordance with the methods of the invention. In a specific embodiment, lymphocytes (B and T lymphocytes) are isolated from the synovial fluid sample and used in accordance with the methods of the invention. In another embodiment, monocytes or neutrophils are isolated from the synovial fluid sample and used in accordance with the methods of the invention. Cells isolated from the synovial fluid can be frozen by standard techniques prior to use in the present methods.

5.1.3 Blood

[0209] In one aspect of the invention, a sample of blood is obtained from a subject according to methods well known in the art. A sample of blood may be obtained from a subject having any of the following developmental or disease stages: fetal, normal, mild, osteoarthritic, moderate osteoarthritic, marked osteoarthritic or severe osteoarthritic or from a test individual where it is unknown whether the individual has osteoarthritis, or has a stage of osteoarthritis. In some embodiments, a drop of blood is collected from a simple pin prick made in the skin of a subject. In such embodiments, this drop of blood collected from a pin prick is all that is needed. Blood may be drawn from a subject from any part of the body (e.g., a finger, a hand, a wrist, an arm, a leg, a foot, an ankle, a stomach, and a neck) using techniques known to one of skill in the art, in particular methods of phlebotomy known in the art. In a specific embodiment, venous blood is obtained from a subject and utilized in accordance with the methods of the invention. In another embodiment, arterial blood is obtained and utilized in accordance with the methods of the invention. The composition of venous blood varies according to the metabolic needs of the area of the body it is servicing. In contrast, the composition of arterial blood is consistent throughout the body. For routine blood tests, venous blood is generally used.

[0210] Venous blood can be obtained from the basilic vein, cephalic vein, or median vein. Arterial blood can be obtained from the radial artery, brachial artery or femoral artery. A vacuum tube, a syringe or a butterfly may be used to draw the blood. Typically, the puncture site is cleaned, a tourniquet is applied approximately 3-4 inches above the puncture site, a needle is inserted at about a 15-45 degree angle, and if using a vacuum tube, the tube is pushed into the needle holder as soon as the needle penetrates the wall of the vein. When finished collecting the blood, the needle is removed and pressure is maintained on the puncture site. Usually, heparin or another type of anticoagulant is in the tube or vial that the blood is collected in so that the blood does not clot. When collecting arterial blood, anesthetics can be administered prior to collection.

[0211] The amount of blood collected will vary depending upon the site of collection, the amount required for a method of the invention, and the comfort of the subject. However, an advantage of one embodiment of the present invention is that the amount of blood required to implement the methods of the present invention can be so small that more invasive procedures are not required to obtain the sample. For example, in some embodiments, all that is required is a drop of blood. This drop of blood can be obtained, for example, from a simple pinprick. In some embodiments, any amount of blood is collected that is sufficient to detect the expression of one, two, three, four, five, ten or more genes listed in Table 1. As such, in some embodiments, the amount of blood that is collected is 1 μl or less, 0.5 μl or less, 0.1 μl or less, or 0.01 μl or less. However, the present invention is not limited to such embodiments. In some embodiments more blood is available and in some embodiments, more blood can be used to effect the methods of the present invention. As such, in various specific embodiments, 0.001 ml, 0.005 ml, 0.01 ml, 0.05 ml, 0.1 ml, 0.15 ml, 0.2 ml, 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, 1.5 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml, 15 ml or more of blood is collected from a subject. In another embodiment, 0.001 ml to 15 ml, 0.01 ml to 10 ml, 0.1 ml to 10 ml, 0.1 ml to 5 ml, 1 to 5 ml of blood is collected from a subject.

[0212] In some embodiments of the present invention, blood is stored within a K3/EDTA tube. In another embodiment, one can utilize tubes for storing blood which contain stabilizing agents such as disclosed in U.S. Pat. No. 6,617,170 (which is incorporated herein by reference). In another embodiment the PAXgene™ blood RNA system:provided by PreAnalytiX, a Qiagen/BD company may be used to collect blood. In yet another embodiment, the Tempus™ blood RNA collection tubes, offered by Applied Biosystems may be used. Tempus™ collection tubes provide a closed evacuated plastic tube containing RNA stabilizing reagent for whole blood collection.

[0213] The collected blood collected is optionally but preferably stored at refrigerated temperatures, such 4° C., prior to use in accordance with the methods of the invention. In some embodiments, a portion of the blood sample is used in accordance with the invention at a first instance of time whereas one or more remaining portions of the blood sample is stored for a period of time for later use. This period of time can be an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely. For long term storage, storage methods well known in the art, such as storage at cryo temperatures (e.g. below −60° C.) can be used. In some embodiments, in addition to storage of the blood or instead of storage of the blood, isolated nucleic acid or proteins are stored for a period of time for later use. Storage of such molecular markers can be for an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely.

[0214] In one aspect, whole blood is obtained from a normal individual or from an individual diagnosed with, or suspected of having osteoarthritis according the methods of phlebotomy well known in the art. Whole blood includes blood which can be used directly, and includes blood wherein the serum or plasma has been removed and the RNA or mRNA from the remaining blood sample has been isolated in accordance with methods well known in the art (e.g., using, preferably, gentle centrifugation at 300 to 800×g for 5 to 10 minutes). In a specific embodiment, whole blood (i.e., unseparated blood) obtained from a subject is mixed with lysing buffer (e.g., Lysis Buffer (1 L): 0.6 g EDTA; 1.0 g $KHCO_2$, 8.2 g NH_4Cl adjusted to pH 7.4 (using NaOH)), the sample is centrifuged and the cell pellet retained, and RNA or mRNA extracted in accordance with methods known in the art ("lysed blood") (see for example Sambrook et al.). The use of whole blood is preferred since it avoids the costly and time-consuming need to separate out the cell types within the blood (Kimoto, 1998, Mol. Gen. Genet 258:233-239; Chelly J et al., 1989, Proc. Nat. Acad. Sci. USA 86:2617-2621; Chelly J et al., 1988, Nature 333:858-860).

[0215] In some embodiments of the present invention, whole blood collected from a subject is fractionated (i.e., separated into components). In specific embodiments of the present invention, blood cells are separated from whole blood

collected from a subject using techniques known in the art. For example, blood collected from a subject can be subjected to Ficoll-Hypaque (Pharmacia) gradient centrifugation. Such centrifugation separates erythrocytes (red blood cells) from various types of nucleated cells and from plasma. In particular, Ficoll-Hypaque gradient centrifugation is useful to isolate peripheral blood leukocytes (PBLs) which can be used in accordance with the methods of the invention.

[0216] By way of example but not limitation, macrophages can be obtained as follows. Mononuclear cells are isolated from peripheral blood of a subject, by syringe removal of blood followed by Ficoll-Hypaque gradient centrifugation. Tissue culture dishes are pre-coated with the subject's own serum or with AB+ human serum and incubated at 37° C. for one hour. Non-adherent cells are removed by pipetting. Cold (4° C.) 1 mM EDTA in phosphate-buffered saline is added to the adherent cells left in the dish and the dishes are left at room temperature for fifteen minutes. The cells are harvested, washed with RPMI buffer and suspended in RPMI buffer. Increased numbers of macrophages can be obtained by incubating at 37° C. with macrophage-colony stimulating factor (M-CSF). Antibodies against macrophage specific surface markers, such as Mac-1, can be labeled by conjugation of an affinity compound to such molecules to facilitate detection and separation of macrophages. Affinity compounds that can be used include but are not limited to biotin, photobiotin, fluorescein isothiocyante (FITC), or phycoerythrin (PE), or other compounds known in the art. Cells retaining labeled antibodies are then separated from cells that do not bind such antibodies by techniques known in the art such as, but not limited to, various cell sorting methods, affinity chromatography, and panning.

[0217] Blood cells can be sorted using a using a fluorescence activated cell sorter (FACS). Fluorescence activated cell sorting (FACS) is a known method for separating particles, including cells, based on the fluorescent properties of the particles. See, for example, Kamarch, 1987, Methods Enzymol 151:150-165. Laser excitation of fluorescent moieties in the individual particles results in a small electrical charge allowing electromagnetic separation of positive and negative particles from a mixture. An antibody or ligand used to detect a blood cell antigenic determinant present on the cell surface of particular blood cells is labeled with a fluorochrome, such as FITC or phycoerythrin. The cells are incubated with the fluorescently labeled antibody or ligand for a time period sufficient to allow the labeled antibody or ligand to bind to cells. The cells are processed through the cell sorter, allowing separation of the cells of interest from other cells. FACS sorted particles can be directly deposited into individual wells of microtiter plates to facilitate separation.

[0218] Magnetic beads can be also used to separate blood cells in some embodiments of the present invention. For example, blood cells can be sorted using a using a magnetic activated cell sorting (MACS) technique, a method for separating particles based on their ability to bind magnetic beads (0.5-100 m diameter). A variety of useful modifications can be performed on the magnetic microspheres, including covalent addition of an antibody which specifically recognizes a cell-solid phase surface molecule or hapten. A magnetic field is then applied, to physically manipulate the selected beads. In a specific embodiment, antibodies to a blood cell surface marker are coupled to magnetic beads. The beads are then mixed with the blood cell culture to allow binding. Cells are then passed through a magnetic field to separate out cells having the blood cell surface markers of interest. These cells can then be isolated.

[0219] In some embodiments, the surface of a culture dish may be coated with antibodies, and used to separate blood cells by a method called panning. Separate dishes can be coated with antibody specific to particular blood cells. Cells can be added first to a dish coated with blood cell specific antibodies of interest. After thorough rinsing, the cells left bound to the dish will be cells that express the blood cell markers of interest. Examples of cell surface antigenic determinants or markers include, but are not limited to, CD2 for T lymphocytes and natural killer cells, CD3 for T lymphocytes, CD11a for leukocytes, CD28 for T lymphocytes, CD19 for B lymphocytes, CD20 for B lymphocytes, CD21 for B lymphocytes, CD22 for B lymphocytes, CD23 for B lymphocytes, CD29 for leukocytes, CD14 for monocytes, CD41 for platelets, CD61 for platelets, CD66 for granulocytes, CD67 for granulocytes and CD68 for monocytes and macrophages.

[0220] Whole blood can be separated into cells types such as leukocytes, platelets, erythrocytes, etc. and such cell types can be used in accordance with the methods of the invention. Leukocytes can be further separated into granulocytes and agranulocytes using standard techniques and such cells can be used in accordance with the methods of the invention. Granulocytes can be separated into cell types such as neutrophils, eosinophils, and basophils using standard techniques and such cells can be used in accordance with the methods of the invention. Agranulocytes can be separated into lymphocytes (e.g., T lymphocytes and B lymphocytes) and monocytes using standard techniques and such cells can be used in accordance with the methods of the invention. T lymphocytes can be separated from B lymphocytes and helper T cells separated from cytotoxic T cells using standard techniques and such cells can be used in accordance with the methods of the invention. Separated blood cells (e.g., leukocytes) can be frozen by standard techniques prior to use in the present methods.

[0221] A blood sample that is useful according to the invention is in an amount that is sufficient for the detection of one or more nucleic acid or amino acid sequences according to the invention. In a specific embodiment, a blood sample useful according to the invention is in an amount ranging from 1 μl to 100 ml, preferably 10 μl to 50 ml, more preferably 10 μl to 25 ml and most preferably 10 μl to 1 ml.

5.1.4 RNA Preparation

[0222] In one aspect of the invention, RNA is isolated from an individual in order to measure the RNA products of the biomarkers of the invention. RNA is isolated from cartilage samples from various disease or developmental stages as described herein. Samples can be from a single patient or can be pooled from multiple patients.

[0223] In another aspect, RNA is isolated directly from synovial fluid of persons with various disease or developmental stages of osteoarthritis as described herein. Samples can be from a single patient or can be pooled from multiple patients.

[0224] In another aspect, RNA is isolated directly from blood samples of persons with various disease or developmental stages of osteoarthritis as described herein. Samples can be from a single patient or can be pooled from multiple patients.

[0225] Total RNA is extracted from the cartilage samples according to methods well known in the art. In one embodi-

ment, RNA is purified from cartilage tissue according to the following method. Following removal of a tissue of interest from an individual or patient, the tissue is quick frozen in liquid nitrogen, to prevent degradation of RNA. Upon the addition of a volume of tissue guanidinium solution, tissue samples are ground in a tissuemizer with two or three 10-second bursts. To prepare tissue guanidinium solution (1 L) 590.8 g guanidinium isothiocyanate is dissolved in approximately 400 ml DEPC-treated H_2O. 25 ml of 2 M Tris-Cl, pH 7.5 (0.05 M final) and 20 ml Na_2EDTA (0.01 M final) is added, the solution is stirred overnight, the volume is adjusted to 950 ml, and 50 ml 2-ME is added.

[0226] Homogenized tissue samples are subjected to centrifugation for 10 min at 12,000×g at 12° C. The resulting supernatant is incubated for 2 min at 65° C. in the presence of 0.1 volume of 20% Sarkosyl, layered over 9 ml of a 5.7M CsCl solution (0.1 g CsCl/ml), and separated by centrifugation overnight at 113,000×g at 22° C. After careful removal of the supernatant, the tube is inverted and drained. The bottom of the tube (containing the RNA pellet) is placed in a 50 ml plastic tube and incubated overnight (or longer) at 4° C. in the presence of 3 ml tissue resuspension buffer (5 mM EDTA, 0.5% (v/v) Sarkosyl, 5% (v/v) 2-ME) to allow complete resuspension of the RNA pellet. The resulting RNA solution is extracted sequentially with 25:24:1 phenol/chloroform/isoamyl alcohol, followed by 24:1 chloroform/isoamyl alcohol, precipitated by the addition of 3 M sodium acetate, pH 5.2, and 2.5 volumes of 100% ethanol, and resuspended in DEPC water (Chirgwin et al., 1979, *Biochemistry,* 18:5294).

[0227] Alternatively, RNA is isolated from cartilage tissue according to the following single step protocol. The tissue of interest is prepared by homogenization in a glass teflon homogenizer in 1 ml denaturing solution (4M guanidinium thiosulfate, 25 mM sodium citrate, pH 7.0, 0.1M 2-ME, 0.5% (w/v) N-laurylsarkosine) per 100 mg tissue. Following transfer of the homogenate to a 5-ml polypropylene tube, 0.1 ml of 2 M sodium acetate, pH 4, 1 ml water-saturated phenol, and 0.2 ml of 49:1 chloroform/isoamyl alcohol are added sequentially. The sample is mixed after the addition of each component, and incubated for 15 min at 0-4° C. after all components have been added. The sample is separated by centrifugation for 20 min at 10,000×g, 4° C., precipitated by the addition of 1 ml of 100% isopropanol, incubated for 30 minutes at −20° C. and pelleted by centrifugation for 10 minutes at 10,000×g, 4° C. The resulting RNA pellet is dissolved in 0.3 ml denaturing solution, transferred to a microfuge tube, precipitated by the addition of 0.3 ml of 100% isopropanol for 30 minutes at −20° C., and centrifuged for 10 minutes at 10,000×g at 4° C. The RNA pellet is washed in 70% ethanol, dried, and resuspended in 100-200 μl DEPC-treated water or DEPC-treated 0.5% SDS (Chomczynski and Sacchi, 1987, *Anal. Biochem.,* 162:156).

[0228] Preferably, the cartilage samples are finely powdered under liquid nitrogen and total RNA is extracted using TRIzol® reagent (GIBCO/BRL).

[0229] Alternatively, RNA is isolated from blood by the following protocol. Lysis Buffer is added to blood sample in a ratio of 3 parts Lysis Buffer to 1 part blood (Lysis Buffer (1 L) 0.6 g EDTA; 1.0 g $KHCO_2$, 8.2 g NH_4Cl adjusted to pH 7.4 (using NaOH)). Sample is mixed and placed on ice for 5-10 minutes until transparent. Lysed sample is centrifuged at 1000 rpm for 10 minutes at 4° C., and supernatant is aspirated. Pellet is resuspended in 5 ml Lysis Buffer, and centrifuged again at 1000 rpm for 10 minutes at 4° C. Pelleted cells are homogenized using TRIzol® (GIBCO/BRL) in a ratio of approximately 6 ml of TRIzol® for every 10 ml of the original blood sample and vortexed well. Samples are left for 5 minutes at room temperature. RNA is extracted using 1.2 ml of chloroform per 1 ml of TRIzol®. Sample is centrifuged at 12,000×g for 5 minutes at 4° C. and upper layer is collected. To upper layer, isopropanol is added in ratio of 0.5 ml per 1 ml of TRIzol®. Sample is left overnight at −20° C. or for one hour at −20° C. RNA is pelleted in accordance with known methods, RNA pellet air dried, and pellet resuspended in DEPC treated ddH_2O. RNA samples can also be stored in 75% ethanol where the samples are stable at room temperature for transportation.

[0230] Alternatively, RNA is isolated from synovial fluid using TRIzol® reagent (GIBCO/BRL) as above.

[0231] Purity and integrity of RNA is assessed by absorbance at 260/280 nm and agarose gel electrophoresis followed by inspection under ultraviolet light.

5.2 Biomarkers of the Invention

[0232] In one embodiment, the invention provides biomarkers and biomarker combinations wherein the measure of the level of expression of the product or products of said biomarkers is indicative of the existence of osteoarthritis. In another embodiment, the invention provides biomarkers and biomarker combinations, wherein the measure of the level of expression of the product or products of said biomarkers can be used to diagnose whether an individual has one of two stages of osteoarthritis. In yet another embodiment, the invention provides biomarkers and biomarker combinations, wherein the measure of the level of expression of the product or products of said biomarkers can be used to diagnose an individual as having a specific stage of osteoarthritis as compared with any other stage of osteoarthritis.

[0233] Table 1 provides a list of the gene names and the associated locus link ID for the biomarkers of the invention wherein the measure of the level of expression of the biomarkers, either individually, or in combination can be used to diagnose an individual as having osteoarthritis, diagnose whether an individual has one of two stages of osteoarthritis or diagnose an individual as having a specific stage of osteoarthritis. As would be understood by a person skilled in the art, the locus link ID can be used to determine the sequence of all the RNA transcripts and all of the proteins which correspond to the biomarkers of the invention. Table 2 provides, in one embodiment of the invention, sequences of the RNA transcripts whose sequences can be used to measure the level of expression of the biomarkers of the invention using those techniques known to a person skilled in the art. Table 2 also provides, in one embodiment of the invention, sequences of the proteins which can be used to measure the level of expression of the biomarkers of the invention. The invention thus encompasses the use of those methods known to a person skilled in the art to measure the expression of these biomarkers and combinations of biomarkers for each of the purposes outlined above.

5.3 Combinations of Biomarkers

[0234] In one embodiment, combinations of biomarkers of the present invention includes any combination of 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, 30, 40 or all of the biomarkers listed in Table 1. In another embodiment of the invention, combinations of biomarkers of the present invention include any combination

of the biomarkers selected from the list in FIG. **1** the measurement of expression of the products of which can be used for diagnosing whether an individual has mild osteoarthritis or does not have osteoarthritis. In another embodiment of the invention, combinations of biomarkers of the present invention include any combination of the biomarkers selected from the list in FIG. **2** the measurement of expression of the products of which can be used in diagnosing whether an individual has moderate osteoarthritis or does not have osteoarthritis. In another embodiment of the invention, combinations of biomarkers of the present invention include any combination of the biomarkers selected in FIG. **3**, the measurement of expression of the products of which can be used for use in diagnosing whether an individual has moderate osteoarthritis or has mild osteoarthritis. In another embodiment of the invention, combinations of biomarkers of the present invention include any combination of the biomarkers selected from the list in FIG. **4** the measurement of expression of the products of which can be used for diagnosing whether an individual has marked osteoarthritis or has severe osteoarthritis.

[0235] For instance, the number of possible combinations of a subset m of n genes is described in Feller, *Intro to Probability Theory,* Third Edition, volume 1, 1968, ed. J. Wiley, using the general formula:

$$m!/(n)!(m-n)!$$

[0236] In one embodiment, where n is 2 and m is 19, there are:

$$\frac{19!}{2!(19-2)!} = \frac{19\times18\times17\times16\times15\times14\times13\times12\times 11\times10\times9\times8\times7\times6\times5\times4\times3\times2\times1}{(2\times1)\left(19\times18\times17\times16\times15\times14\times13\times12\times 11\times10\times9\times8\times7\times6\times5\times4\times3\times2\times1\right)}$$

$$= \frac{1.216\ 10^{17}}{7.11\ 10^{14}}$$

$$= 171$$

unique two-gene combinations. The measurement of the gene expression of each of these two-gene combinations can independently be used to determine whether a patient has osteoarthritis. In another specific embodiment in which m is 19 and n is three, there are 19!/3!(19−3)! unique three-gene combinations. Each of these unique three-gene combinations can independently serve as a model for determining whether a patient has osteoarthritis.

5.4 Particularly Useful Combinations of Biomarkers

[0237] Although all of the combinations of the biomarkers of the invention are useful for diagnosing OA, the invention further provides a means of selecting those combinations of biomarkers particularly useful for each of the following (a) diagnosing individuals as having osteoarthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). The invention further provides a method of evaluating the combinations identified for each of the utilities described above.

[0238] In order to identify useful combinations of biomarkers a mathematical model of the invention is used to test each of the possible combinations of the biomarkers of the invention for each combinations ability to separate as between the two (e.g. binary models such as logistic regression) or more (e.g. models such as neural networks) phenotypic traits of a training population used for input into the model. The phenotypic traits of the training population used for input into the model are phenotypic traits for use in (a) diagnosing as OA; (b) diagnosed as having mild OA (c) diagnosed as having moderate OA (d) diagnosed as having severe OA (e) does not have OA. The phenotypic traits of the training population used for input into the mathematical model, and the model used, will determine the utility of the combinations generated by the model as a diagnostic for one of the following a) diagnosing individuals as having osteoarthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). The result of the choice of phenotypic traits of the training population used for entry into the mathematical model is described more thoroughly below.

[0239] The mathematical model generated can be subsequently evaluated by determining the ability of the model to correctly call each individual for one of the two (or more) phenotypic traits of the population used for input into the model. In a preferred embodiment, the individuals of the training population used to derive the model are different from the individuals of the training population used to test the model. As would be understood by a person skilled in the art, this allows one to predict the ability of the combinations as to their ability to properly characterize an individual whose phenotypic characterization is unknown.

[0240] The data which is input into the mathematical model can be any data which is representative of the expression level of the product of the biomarkers being evaluated. Mathematical models useful in accordance with the invention include those using both supervised or unsupervised learning. In a preferred embodiment of the invention, the mathematical model chosen uses supervised learning in conjunction with a "training population" evaluate each of the possible combination of biomarkers of the invention. In one embodiment of the invention, the mathematical model used is selected from the following: a regression model, a logistic regression model, a neural network, a clustering model, principal component analysis, nearest neighbour classifier analysis, linear discriminant analysis, quadratic discriminant analysis, a support vector machine, a decision tree, a genetic algorithm, classifier optimization using bagging, classifier optimization using boosting, classifier optimization using the Random Subspace Method, a projection pursuit, and weighted voting. In a preferred embodiment, a logistic regression model is used. In another preferred embodiment, a neural network model is used.

[0241] The resulting mathematical model can be used to diagnosis an unknown or test individual for the phenotypic trait used for input into the model. In a preferred embodiment, the diagnosis result from equations generated by logistic regression to answer one of the following questions: (a) does an individual have osteoarthritis, (b) which of two stages of osteoarthritis does an individual have (wherein not having osteoarthritis is considered a stage of OA) and (c) does an individual have a specific stage of osteoarthritis. In yet another embodiment of the invention, the answer to any of the questions above may be an answer of non determinable.

[0242] In one preferred embodiment of the invention, each model is evaluated for its ability to properly characterize each individual of the training population using those methods known to a person skilled in the art. For example one can

evaluate the model using cross validation Leave One out Cross Validation, n-fold cross validation, jackknife analysis using standard statistical methods and disclosed. In an even more preferred embodiment of the invention, each model is evaluated for its ability to properly characterize those individuals of the training population which were not used to generate the model.

[0243] In one embodiment, the method used to evaluate the model for its ability to properly characterize each individual of the training population is a method which evaluates the models sensitivity (TPF, true positive fraction) and 1-specificity (TNF, true negative fraction). In a preferred embodiment, the method used to test the model is Receiver Operating Characteristic ("ROC") which provides several parameters to evaluate both the sensitivity and specificity of the diagnostic result of the equation generated. In a particularly preferred embodiment, the ROC area (area under the curve) is used to evaluate the equations. In a preferred embodiment, an ROC area greater than 0.5, 0.6, 0.7, 0.8, 0.9 is preferred. A perfect ROC area score of 1.0 on the other hand indicates with both 100% sensitivity and 100% specificity.

[0244] As would be understood by a person skilled in the art, the utility of the combinations and equations determined by a mathematical model will depend upon the phenotypes of the populations used to generate the data for input into the model. Examples of specific embodiments are described more thoroughly herein.

5.5 Populations for Input into the Mathematical Models

[0245] In some embodiments, the reference or training population includes between 10 and 30 subjects. In another embodiment the training population contains between 30-50 subjects. In still other embodiments, the reference population includes two or more populations each containing between 50 and 100, 100 and 500, between 500 and 1000, or more than lilt subjects.

[0246] The mathematical model used will also determine how many phenotypic traits can be contained within the reference population. For example some models (binary mathematical models) can only identify biomarker combinations useful to differentiate as between two phenotypic trait (e.g. logistic regression). In other models, biomarker combinations which are useful to differentiate as between three or more phenotypic traits can be used (e.g. neural networks). In another embodiment of the invention, one can use combinations of binary decisions using binary models to differentiate multiple groups.

5.6 Populations to Identify Biomarkers for Diagnosis of Osteoarthritis for Input into Binary Mathematical Models

[0247] For example, in order to identify those biomarkers which are useful in diagnosing an individual as having osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of all of the mRNA products of the biomarkers of Table 1 are used from a population of individuals having osteoarthritis, and a second population of individuals not having osteoarthritis are used. For purposes of characterizing the populations as having or not having osteoarthritis, any traditional method of OA diagnosis can be used. In a preferred embodiment, the scoring method of Marshall as described herein is used. In one embodiment of the invention, a population of individuals considered to have osteoarthritis includes any individual who is scored as having mild OA, moderate OA, marked OA or severe OA. Similarly population of individuals not having OA are chosen according to similar methods. In a preferred embodiment, the phenotypic characteristics of the two populations used in the training set are similar but for having or not having OA. In another preferred embodiment, the two populations are at least age, sex and BMI (body mass index) matched.

[0248] In another embodiment, in order to identify those biomarkers which are useful in diagnosing an individual as having osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of one or more of the mRNA products of the biomarkers of Table 1 resulting from a population of individuals having osteoarthritis, and the second population of individuals does not have osteoarthritis are used.

[0249] In another embodiment, in order to identify those biomarkers which are useful in diagnosing an individual as having osteoarthritis, or not having osteoarthritis, data reflective of the level of expression one or more of the mRNA products of the biomarkers of Table 1 as noted in Table 2 resulting from a population of individuals having osteoarthritis, and a second population of individuals not having osteoarthritis are used.

[0250] In another embodiment, in order to identify those biomarkers which are useful in diagnosing an individual as having osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of all of the RNA products of the biomarkers of Table 1 which are expressed in blood resulting from a population of individuals having osteoarthritis, and a second population of individuals not having osteoarthritis are used.

5.7 Populations to Identify Biomarkers for Differentiation as Between Stages of Osteoarthritis Using Binary Mathematical Models

[0251] In one embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having mild osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of all of the mRNA products of the biomarkers of Table 1 resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the a second population of individuals not having osteoarthritis are used.

[0252] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having mild osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of one or more of the mRNA products of the biomarkers of Table 1 from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0253] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having mild osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of the mRNA products from the biomarkers of Table 1 as disclosed in Table 2 from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0254] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having mild osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of the mRNA products of the biomarkers of Table 1 which are expressed in blood resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0255] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having mild osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of all of the mRNA products of the biomarkers of FIG. **1** resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0256] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having moderate osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of all of the product of the mRNA of the biomarkers of Table 1 resulting from a population of individuals identified by any traditional method of OA diagnosis as having moderate osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0257] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having moderate osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of one or more of the mRNA products of the biomarkers of Table 1 from a population of individuals identified by any traditional method of OA diagnosis as having moderate osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0258] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having moderate osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of the mRNA products of the biomarkers of Table 1 which are expressed in blood resulting from a population of individuals identified by any traditional method of OA diagnosis as having moderate osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0259] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having moderate osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of the mRNA products of the biomarkers of Table 1 as reflected in Table 2 resulting from a population of individuals identified by any traditional method of OA diagnosis as having moderate osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0260] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in diagnosing an individual as having moderate osteoarthritis, or not having osteoarthritis, data reflective of the level of expression of the mRNA products of the biomarkers of FIG. **2** resulting from a population of individuals identified by any traditional method of OA diagnosis as having moderate osteoarthritis, and the second population of individuals not having osteoarthritis are used.

[0261] Similarly, combinations of biomarkers useful to differentiate between individuals as having marked OA or no OA; having severe OA or no OA; mild OA or moderate OA; mild OA or marked OA, mild OA or severe OA; moderate OA or marked OA; moderate OA or severe OA; and marked OA or severe OA can be identified using similar methods to those outlined above.

5.8 Populations to Identify Biomarkers for Diagnosing Stage Specific OA for Input into Binary Mathematical Models

[0262] In one embodiment of the invention, in order to identify those combinations of biomarkers which are useful in identifying whether an individual has mild OA as compared to any other stage of OA (ie identifying biomarkers to diagnose the stage of an individual's OA) data reflective of the level of expression of the mRNA products of each of the biomarkers of Table 1 resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals which is a combination of individuals having moderate OA, individuals having marked OA, individuals having severe OA, and individuals not having OA are used. Preferably individuals as between both populations are matched for age, sex and BMI.

[0263] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in identifying whether an individual has mild OA as compared to any other stage of OA (ie identifying biomarkers to diagnose an individual with a specific stage of OA) data reflective of the level of expression of each of the possible variants of biomarkers of Table 1 resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals which is a combination of individuals having moderate OA, individuals having marked OA, individuals having severe OA, and individuals not having OA are used. Preferably individuals as between both populations are matched for age, sex and BMI.

[0264] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in identifying whether an individual has mild OA as compared to any other stage of OA (ie identifying biomarkers to diagnose an individual with a specific stage of OA) data reflective of the level of expression each of the possible variants of biomarkers of Table 1 as selected from the list provided in Table 2 resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals which is a combination of individuals having moderate OA, individuals having marked OA, individuals having severe OA, and individuals not having OA are used. Preferably individuals as between both populations are matched for age, sex and BMI.

[0265] In another embodiment of the invention, in order to identify those combinations of biomarkers which are useful in identifying whether an individual has mild OA as compared to any other stage of OA (ie identifying biomarkers to diagnose an individual with a specific stage of OA) data reflective of the level of expression of those variants of biomarkers of Table 1 expressed in blood resulting from a population of individuals identified by any traditional method of OA diagnosis as having mild osteoarthritis, and the second population of individuals which is a combination of individu-

als having moderate OA, individuals having marked OA, individuals having severe OA, and individuals not having OA are used. Preferably individuals as between both populations are matched for age, sex and BMI.

[0266] Similarly, combinations of biomarkers useful to differentiate between individuals as having moderate OA as compared with any other stage of OA; marked OA as compared with any other stage of OA; and severe OA as compared with any other stage of OA can be identified using data from populations using similar methods to those outlined above.

5.9 Populations to Identify Biomarkers for Diagnosing Stage Specific OA for Input into Non Binary Mathematical Models

[0267] In one embodiment of the invention, in order to identify those combinations of biomarkers which are useful in identifying whether an individual has mild OA as compared to any other stage of OA, data reflective of the level of expression of each of the biomarkers of Table 1 resulting from the following populations of individuals are used (a) individuals having mild OA (b) individuals having moderate OA (c) individuals having marked OA (d) individuals having severe OA. The populations are made up of individuals identified by any traditional method of OA diagnosis but preferably the scoring method of Marshall (supra) is used. Preferably individuals as between the populations are matched for age, sex and BMI.

[0268] Similarly, combinations of biomarkers useful to characterize an individual as having moderate OA as compared with any other stage of OA; marked OA as compared with any other stage of OA; and severe OA as compared with any other stage of OA can be identified using similar methods to those outlined above.

5.10 Regression Models

[0269] In some embodiments the expression data for some or all of the possible combination of biomarkers identified in the present invention are used in a regression model, preferably a logistic regression model. Such a regression model will determine one or more equations for each possible combination of biomarkers, each equation providing a coefficient for each of the biomarkers represented by the model.

[0270] In general, the multiple regression equation of interest can be written

$$Y=\alpha+\beta_1X_1+\beta_2X_2+\ldots+\beta_kX_k+\epsilon$$

where Y, the dependent variable, is presence (when Y is positive) or absence (when Y is negative) of the biological feature (e.g., absence/presence/stage of osteoarthritis) associated with the first subgroup. This model says that the dependent variable Y depends on k explanatory variables (the measured characteristic values for the k select genes from subjects in the first and second subgroups in the training data set), plus an error term that encompasses various unspecified omitted factors. In the above-identified model, the parameter β_1 gauges the effect of the first explanatory variable X_1 on the dependent variable Y, holding the other explanatory variables constant. Similarly, β2 gives the effect of the explanatory variable X_2 on Y, holding the remaining explanatory variables constant.

[0271] The logistic regression model is a non-linear transformation of the linear regression. The logistic regression model is termed the "logit" model and can be expressed as

$$\ln[p/(1-p)]=\alpha+\beta_1X_1+\beta_2X_2+\ldots+\beta_kX_k+\epsilon \text{ or}$$

$$[p/(1-p)]=\exp^{\alpha}\exp^{\beta_1X_1}\exp^{\beta_2X_1}\times\ldots\times\exp^{\beta_kX_k}\exp^{\epsilon}$$

where,

[0272] ln is the natural logarithm, $\log^{exp}$, where exp=2.71828 . . . ,

[0273] p is the probability that the event Y occurs, p(Y=1),

[0274] p/(1−p) is the "odds ratio",

[0275] ln[p/(1−p)] is the log odds ratio, or "logit", and

[0276] all other components of the model are the same as the general regression equation described above. It will be appreciated by those of skill in the art that the term for α and ϵ can be folded into the same constant. Indeed, in preferred embodiments, a single term is used to represent α and ϵ. The "logistic" distribution is an S-shaped distribution function. The logit distribution constrains the estimated probabilities (p) to lie between 0 and 1.

[0277] In some embodiments of the present invention, the logistic regression model is fit by maximum likelihood estimation (MLE) In other words, the coefficients (e.g., α, β_1, β_2, . . .) are determined by maximum likelihood. A likelihood is a conditional probability (e.g., P(Y|X), the probability of Y given X). The likelihood function (L) measures the probability of observing the particular set of dependent variable values (Y_1, Y_2, . . . , Y_n) that occur in the sample data set. It is written as the probability of the product of the dependent variables:

$$L=\text{Prob}(Y_1*Y_2***Y_n)$$

The higher the likelihood function, the higher the probability of observing the Ys in the sample. MLE involves finding the coefficients (α, β_1, β_2 . . .) that makes the log of the likelihood function (L<0) as large as possible or −2 times the log of the likelihood function (−2LL) as small as possible. In MLE, some initial estimates of the parameters α, β_1, β_2, . . . are made. Then the likelihood of the data given these parameter estimates is computed. The parameter estimates are improved the likelihood of the data is recalculated. This process is repeated until the parameter estimates do not change much (for example, a change of less than 0.01 or 0.001 in the probability). Examples of logistic regression and fitting logistic logistic regression models are found in Hastie, *The Elements of Statistical Learning,* Springer, New York, 2001, pp. 95-100 which is incorporated herein in its entirety.

5.11 Neural Networks

[0278] In another embodiment, the expression measured for each of the biomarkers of the present invention can be used to train a neural network. A neural network is a two-stage regression or classification model. A neural network has a layered structure that includes a layer of input units (and the bias) connected by a layer of weights to a layer of output units. For regression, the layer of output units typically includes just one output unit. However, neural networks can handle multiple quantitative responses in a seamless fashion. As such a neural network can be applied to allow identification of biomarkers which differentiate as between more than two populations. In one specific example, a neural network can be trained using expression data from the biomarkers in Table 1 to identify those combinations of biomarkers which are specific for a stage of osteoarthritis (e.g mild osteoarthritis) as compared with any other stage of osteoarthritis wherein not having osteoarthritis can be considered a stage of osteoarthritis. As a result, the trained neural network can be used to directly identify combination of biomarkers useful as stage specific biomarkers. In some embodiments, the back-propagation

neural network (see, for example Abdi, 1994, "A neural network primer", J. Biol System. 2, 247-283) containing a single hidden layer of ten neurons (ten hidden units) found in EasyNN-Plus version 4.0g software package (Neural Planner Software Inc.) is used

[0279] Neural networks are described in Duda et al., 2001, *Pattern Classification,* Second Edition, John Wiley & Sons, Inc., New York; and Hastie et al., 2001, *The Elements of Statistical Learning,* Springer-Verlag, New York which is incorporated herein in its entirety.

5.12 Other Mathematical Models

[0280] The pattern classification and statistical techniques described above are merely examples of the types of models that can be used to construct a model for OA classification, for example clustering as described on pages 211-256 of Duda and Hart, *Pattern Classification and Scene Analysis,* 1973, John Wiley & Sons, Inc., New York, incorporated herein by reference in its entirety; Principal component analysis, (see for Jolliffe, 1986, *Principal Component* Analysis, Springer, New York, incorporated herein by reference); nearest neighbour classifier analysis, (see for example Duda, *Pattern Classification,* Second Edition, 2001, John Wiley & Sons, Inc; and Hastie, 2001, *The Elements of Statistical Learning,* Springer, New York); linear discriminant analysis, (see for example Duda, *Pattern Classification,* Second Edition, 2001, John Wiley & Sons, Inc; and Hastie, 2001, *The Elements of Statistical Learning,* Springer, New York; Venables & Ripley, 1997, *Modern Applied Statistics with s-plus,* Springer, New York); Support Vector Machines (see, for example, Cristianini and Shawe-Taylor, 2000, *An introduction to Support Vector Machines,* Cambridge University Press, Cambridge, Boser et al., 1992, "A training algorithm for optimal margin classifiers, in *Proceedings of the 5th Annual ACM Workshop on Computational Learning Theory,* ACM Press, Pittsburgh, Pa., pp. 142-152; Vapnik, 1998, *Statistical Learning Theory,* Wiley, New York, incorporated herein by reference.)

5.13 Products of the Biomarkers of the Invention

[0281] As would be understood by a person skilled in the art, the identification of one or more of a combination of biomarkers which are differentially expressed during OA and or stages of OA allows the diagnosis of OA and stages of OA by measurement of the expression of the products of the biomarkers (gene) in an individual.

[0282] The products of each of the biomarkers of the invention includes both RNA and protein. RNA products of the biomarkers of the invention include populations of hnRNA, mRNA, and one or more spliced variants of mRNA. To practice the invention, measurement of one or more of the populations of the RNA products of the biomarkers of the invention can be used for purposes of diagnosis. More particularly, measurement of those populations of RNA products of the biomarkers which are differentially expressed during OA, or during a stage of OA are useful for purposes of diagnosis and are encompassed herein.

[0283] In one embodiment of the invention, the RNA products of the biomarkers of the invention which are measured is the population of RNA products including the hnRNA, the mRNA, and all of the spliced variants of the mRNA. In another embodiment, the RNA products of the biomarkers of the invention which are measured is the population of mRNA. In another embodiment of the invention the RNA products of the biomarkers of the invention which are measured is the population of mRNA which is, expressed in blood. In yet another embodiment of the invention, RNA products of the biomarkers of the invention which are measured is the population of one or more spliced variants of the mRNA. In yet another embodiment of the invention, RNA products of the biomarkers of the invention which are measured is the population of one or more spliced variants of the mRNA which are expressed in blood. In yet another embodiment of the invention, RNA products of the biomarkers of the invention are those products which are listed in Table 2.

[0284] Protein products of the biomarkers of the invention are also included within the scope of the invention and include the entire population of protein arising from a biomarker of the invention. As would be understood by a person skilled in the art, the entire population of proteins arising from a biomarker of the invention include proteins, protein variants arising from spliced mRNA variants, and post translationally modified proteins. To practice the invention, measurement of one or more of the populations of the protein products of the biomarkers of the invention can be used for purposes of diagnosis. More particularly, measurement of those populations of protein products of the biomarkers which are differentially expressed during OA, or during a stage of OA are useful for purposes of diagnosis and are encompassed herein.

[0285] In one embodiment of the invention the protein products of the biomarkers of the invention which are measured is the entire population of protein products translated from the RNA products of the biomarkers of the invention. In another embodiment, the protein products of the biomarkers of the invention are those protein products which are expressed in blood. In yet another embodiment of the invention, the protein products of the biomarkers of the invention are one or more of the protein products translated from one or more of the mRNA spliced variants. In yet another embodiment of the invention, the protein products of the biomarkers of the invention are one or more of the protein products translated from one or more of the mRNA spliced variants expressed in blood. In yet another embodiment of the invention, protein products of the biomarkers of the invention are those products which are listed in Table 2.

5.14 Use of the Combinations Identified to Diagnose

[0286] The invention teaches the ability to identify useful combinations of biomarkers for the purpose of diagnosing OA, differentiating as between stages of OA and diagnosing a particular stage of OA. Data representative of the RNA or protein products of the biomarkers of the invention is input into a model of the invention so as to identify particularly useful combinations. The diagnostic purpose for which the combination is used is dependant upon the phenotypic traits of the input population as described herein. The combinations identified can be used with traditional techniques for measuring the level of expression of the RNA and protein products of the combinations to determine whether the pattern of expression as between the individual tested and one or more individuals from a control population (wherein the control population would include subpopulations with phenotypic subtraits as defined by the populations input into the model and can include data representative of the control population). In a preferred embodiment, one would use the model generated so as to diagnose an individual, e.g., by the measure of the level of expression of the RNA products of the biomarkers of the invention in a test individual for input into the model

generated to identify the combination to determine a diagnosis as defined by the model. In a preferred embodiment, the same method is used to generate the expression data used to generate the mathematical model as is used to diagnose the test individual.

5.15 Use of the Combinations Identified to Monitor Progression or Regression of OA

[0287] The invention teaches the ability to identify useful combinations of biomarkers for the purposes of diagnosing an individual as having a particular stage of OA. Having identified combinations which diagnose an individual with a particular stage of OA (wherein the stage can be any stage in accordance with known staging methods and includes subclassifications of the Marshall method wherein the stages are defined by the Marshall score. In one embodiment, there are five stages including (a) normal, (b) mild (c) moderate (d) marked (e) severe. It would be understood by a person skilled in the art that combinations which are diagnostic for a specific stage are useful in determining whether an individual has progressed or regressed with regards to the severity of their OA, for example, in response to treatment. For example, an individual can be diagnosed as having a particular stage of OA prior to treatment using one or more of the combinations identified as diagnosing a particular stage of OA. Subsequent to treatment the individual could again be diagnosed using one or more combinations identified as diagnosing a particular stage of OA. In the event that the individual can no longer be identified the stage prior to treatment, this may in itself suggest treatment is effective. In addition, the treatment may lead to regression of the stage of OA such that the individual now is diagnosed with a lesser degree of OA, or the treatment may have progressed the disease such that the individual is now diagnosed with a more severe stage of OA. As such, one or more of the combinations identified as specific to diagnosing a stage of OA is useful so as to monitor progression or regression OA or so as to monitor response to treatment.

5.16 Polynucleotides Used to Measure the Products of the Biomarkers of the Invention

[0288] As a means of measuring the expression of the RNA products of the biomarkers of the invention, one can use one or more of the following as would be understood by a person skilled in the art in combination with one or more methods to measure RNA expression in a sample of the invention: oligonucleotides, cDNA, DNA, RNA, PCR products, synthetic DNA, synthetic RNA, or other combinations of naturally occurring of modified nucleotides which specifically hybridize to one or more of the RNA products of the biomarker of the invention. In another specific embodiment, the oligonucleotides, cDNA, DNA, RNA, PCR products, synthetic DNA, synthetic RNA, or other combinations of naturally occurring of modified nucleotides oligonucleotides which selectively hybridize to one or more of the RNA products of the biomarker of the invention are used. In a preferred embodiment, the oligonucleotides, cDNA, DNA, RNA, PCR products, synthetic DNA, synthetic RNA, or other combinations of naturally occurring of modified nucleotides oligonucleotides which both specifically and selectively hybridize to one or more of the RNA products of the biomarker of the invention are used.

[0289] In one embodiment of the invention, the polynucleotide used to measure the RNA products of the invention can be used as nucleic acid members. Nucleic acid members can be stably associated with a solid support to comprise an array according to one aspect of the invention. The length of a nucleic acid member can range from 8 to 1000 nucleotides in length and are chosen so as to be specific for the RNA products of the biomarkers of the invention. In one embodiment, these members are selective for RNA products of the biomarkers of the invention. In yet another embodiment these members are selective for the mRNA products of the biomarkers of the invention. In a preferred embodiment, these members are selective for all of the variants of the mRNA products of the biomarkers of the invention. In yet another preferred embodiment, these members are selective for one or more variants of the mRNA products of the biomarkers of the invention. The nucleic acid members may be single or double stranded, and/or may be oligonucleotides or PCR fragments amplified from cDNA. Preferably oligonucleotides are approximately 20-30 nucleotides in length. ESTs are preferably 100 to 600 nucleotides in length. It will be understood to a person skilled in the art that one can utilize portions of the expressed regions of the biomarkers of the invention as a probe on the array. More particularly oligonucleotides complementary to the genes of the invention and or cDNA or ESTs derived from the genes of the invention are useful. For oligonucleotide based arrays, the selection of oligonucleotides corresponding to the gene of interest which are useful as probes is well understood in the art. More particularly it is important to choose regions which will permit hybridization to the target nucleic acids. Factors such as the Tm of the oligonucleotide, the percent GC content, the degree of secondary structure and the length of nucleic acid are important factors. See for example U.S. Pat. No. 6,551,784.

5.17 Techniques to Measure the RNA Products of the Biomarkers of the Invention Array Hybridization

[0290] In some embodiments of the invention the polynucleotides capable of specifically and/or selectively hybridizing to RNA products of the biomarkers of the invention can be spotted onto an array for use in the invention. In one embodiment, the array consists of sequences of between 10-1000 nucleotides in length capable of hybridizing to one or more of the products of each of the biomarkers of the invention as disclosed in Table 1.

[0291] The target nucleic acid samples that are hybridized to and analyzed with an array of the invention are preferably from human cartilage, blood or synovial fluid. A limitation for this procedure lies in the amount of RNA available for use as a target nucleic acid sample. Preferably, at least 1 microgram of total RNA is obtained for use according to this invention. Lesser quantities of RNA can be used in combination with PCR and primers directed to the mRNA subspecies (e.g. poly T oligonucleotides).

Construction of a Nucleic Acid Array

[0292] In the subject methods, an array of nucleic acid members stably associated with the surface of a substantially solid support is contacted with a sample comprising target nucleic acids under hybridization conditions sufficient to produce a hybridization pattern of complementary nucleic acid members/target complexes in which one or more complementary nucleic acid members at unique positions on the array specifically hybridize to target nucleic acids. The iden-

tity of target nucleic acids which hybridize can be determined with reference to location of nucleic acid members on the array.

[0293] The nucleic acid members may be produced using established techniques such as polymerase chain reaction (PCR) and reverse transcription (RT). These methods are similar to those currently known in the art (see e.g., *PCR Strategies,* Michael A. Innis (Editor), et al. (1995) and *PCR: Introduction to Biotechniques Series,* C. R. Newton, A. Graham (1997)). Amplified nucleic acids are purified by methods well known in the art (e.g., column purification or alcohol precipitation). A nucleic acid is considered pure when it has been isolated so as to be substantially free of primers and incomplete products produced during the synthesis of the desired nucleic acid. Preferably, a purified nucleic acid will also be substantially free of contaminants which may hinder or otherwise mask the specific binding activity of the molecule.

[0294] An array, according to one aspect of the invention, comprises a plurality of nucleic acids attached to one surface of a solid support at a density exceeding 20 different nucleic acids/cm^2, wherein each of the nucleic acids is attached to the surface of the solid support in a non-identical pre-selected region (e.g. a microarray). Each associated sample on the array comprises a nucleic acid composition, of known identity, usually of known sequence, as described in greater detail below. Any conceivable substrate may be employed in the invention.

[0295] In one embodiment, the nucleic acid attached to the surface of the solid support is DNA. In a preferred embodiment, the nucleic acid attached to the surface of the solid support is cDNA or RNA. In another preferred embodiment, the nucleic acid attached to the surface of the solid support is cDNA synthesized by polymerase chain reaction (PCR). Preferably, a nucleic acid member in the array, according to the invention, is at least 10, 25 or 50 nucleotides in length. In one embodiment, a nucleic acid member is at least 150 nucleotides in length. Preferably, a nucleic acid member is less than 1000 nucleotides in length. More preferably, a nucleic acid member is less than 500 nucleotides in length.

[0296] In the arrays of the invention, the nucleic acid compositions are stably associated with the surface of a support, where the support may be a flexible or rigid solid support. By "stably associated" is meant that each nucleic acid member maintains a unique position relative to the solid support under hybridization and washing conditions. As such, the samples are non-covalently or covalently stably associated with the support surface. Examples of non-covalent association include non-specific adsorption, binding based on electrostatic interactions (e.g., ion pair interactions), hydrophobic interactions, hydrogen bonding interactions, specific binding through a specific binding pair member covalently attached to the support surface, and the like. Examples of covalent binding include covalent bonds formed between the nucleic acids and a functional group present on the surface of the rigid support (e.g., —OH), where the functional group may be naturally occurring or present as a member of an introduced linking group, as described in greater detail below

[0297] The amount of nucleic acid present in each composition will be sufficient to provide for adequate hybridization and detection of target nucleic acid sequences during the assay in which the array is employed. Generally, the amount of each nucleic acid member stably associated with the solid support of the array is at least about 0.001 ng, preferably at least about 0.02 ng and more preferably at least about 0.05 ng, where the amount may be as high as 1000 ng or higher, but will usually not exceed about 20 ng. Preferably multiple samples corresponding to a single gene are spotted onto the array so as to ensure statistically significant results. Where the nucleic acid member is "spotted" onto the solid support in a spot comprising an overall circular dimension, the diameter of the "spot" will generally range from about 10 to 5,000 μm, usually from about 20 to 2,000 μm and more usually from about 100 to 200 μm.

[0298] Control nucleic acid members may be present on the array including nucleic acid members comprising oligonucleotides or nucleic acids corresponding to genomic DNA, housekeeping genes, vector sequences, plant nucleic acid sequence, negative and positive control genes, and the like. Control nucleic acid members are calibrating or control genes whose function is not to tell whether a particular "key" gene of interest is expressed, but rather to provide other useful information, such as background or basal level of expression.

[0299] Other control nucleic acids are spotted on the array and used as target expression control nucleic acids and mismatch control nucleotides to monitor non-specific binding or cross-hybridization to a nucleic acid in the sample other than the target to which the probe is directed. Mismatch probes thus indicate whether a hybridization is specific or not. For example, if the target is present, the perfectly matched probes should be consistently brighter than the mismatched probes. In addition, if all control mismatches are present, the mismatch probes are used to detect a mutation.

Solid Substrate

[0300] An array according to the invention comprises either a flexible or rigid substrate. A flexible substrate is capable of being bent, folded or similarly manipulated without breakage. Examples of solid materials which are flexible solid supports with respect to the present invention include membranes, e.g., nylon, flexible plastic films, and the like. By "rigid" is meant that the support is solid and does not readily bend, i.e., the support is not flexible. As such, the rigid substrates of the subject arrays are sufficient to provide physical support and structure to the associated nucleic acids present thereon under the assay conditions in which the array is employed, particularly under high throughput handling conditions.

[0301] The substrate may be biological, non-biological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, beads, containers, capillaries, pads, slices, films, plates, slides, chips, etc. The substrate may have any convenient shape, such as a disc, square, sphere, circle, etc. The substrate is preferably flat or planar but may take on a variety of alternative surface configurations. The substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SIN_4, modified silicon, or any one of a wide variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, polycarbonate, or combinations thereof. Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure.

[0302] In a preferred embodiment the substrate is flat glass or single-crystal silicon. According to some embodiments, the surface of the substrate is etched using well-known techniques to provide for desired surface features. For example, by way of formation of trenches, v-grooves, mesa structures,

or the like, the synthesis regions may be more closely placed within the focus point of impinging light, be provided with reflective "mirror" structures for maximization of light collection from fluorescent sources, etc.

[0303] Surfaces on the solid substrate will usually, though not always, be composed of the same material as the substrate. Alternatively, the surface may be composed of any of a wide variety of materials, for example, polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, membranes, or any of the above-listed substrate materials. In some embodiments the surface may provide for the use of caged binding members which are attached firmly to the surface of the substrate. Preferably, the surface will contain reactive groups, which are carboxyl, amino, hydroxyl, or the like. Most preferably, the surface will be optically transparent and will have surface Si—OH functionalities, such as are found on silica surfaces.

[0304] The surface of the substrate is preferably provided with a layer of linker molecules, although it will be understood that the linker molecules are not required elements of the invention. The linker molecules are preferably of sufficient length to permit nucleic acids of the invention and on a substrate to hybridize to other nucleic acid molecules and to interact freely with molecules exposed to the substrate.

[0305] Often, the substrate is a silicon or glass surface, (poly)tetrafluoroethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, a charged membrane, such as nylon 66 or nitrocellulose, or combinations thereof. In a preferred embodiment, the solid support is glass. Preferably, at least one surface of the substrate will be substantially flat. Preferably, the surface of the solid support will contain reactive groups, including, but not limited to, carboxyl, amino, hydroxyl, thiol, or the like. In one embodiment, the surface is optically transparent. In a preferred embodiment, the substrate is a poly-lysine coated slide or Gamma amino propyl silane-coated Corning Microarray Technology-GAPS or CMT-GAP2 coated slides.

[0306] Any solid support to which a nucleic acid member may be attached may be used in the invention. Examples of suitable solid support materials include, but are not limited to, silicates such as glass and silica gel, cellulose and nitrocellulose papers, nylon, polystyrene, polymethacrylate, latex, rubber, and fluorocarbon resins such as TEFLON™.

[0307] The solid support material may be used in a wide variety of shapes including, but not limited to slides and beads. Slides provide several functional advantages and thus are a preferred form of solid support. Due to their flat surface, probe and hybridization reagents are minimized using glass slides. Slides also enable the targeted application of reagents, are easy to keep at a constant temperature, are easy to wash and facilitate the direct visualization of RNA and/or DNA immobilized on the solid support. Removal of RNA and/or DNA immobilized on the solid support is also facilitated using slides.

[0308] The particular material selected as the solid support is not essential to the invention, as long as it provides the described function. Normally, those who make or use the invention will select the best commercially available material based upon the economics of cost and availability, the expected application requirements of the final product, and the demands of the overall manufacturing process.

Spotting Method

[0309] In one aspect, the invention provides for arrays where each nucleic acid member comprising the array is spotted onto a solid support.

[0310] Preferably, spotting is carried out as follows. PCR products (~40 ul) of cDNA clones from osteoarthritis, fetal or normal cartilage cDNA libraries, in the same 96-well tubes used for amplification, are precipitated with 4 ul (1/10 volume) of 3M sodium acetate (pH 5.2) and 100 ul (2.5 volumes) of ethanol and stored overnight at −20° C. They are then centrifuged at 3,300 rpm at 4° C. for 1 hour. The obtained pellets are washed with 50 ul ice-cold 70% ethanol and centrifuged again for 30 minutes. The pellets are then air-dried and resuspended well in 20 ul 3×SSC or in 50% dimethylsulfoxide (DMSO) overnight. The samples are then spotted, either singly or in duplicate, onto slides using a robotic GMS 417 or 427 arrayer (Affymetrix, Calif.).

[0311] The boundaries of the spots on the microarray may be marked with a diamond scriber (as the spots become invisible after post-processing). The arrays are rehydrated by suspending the slides over a dish of warm particle free ddH_20 for approximately one minute (the spots will swell slightly but will not run into each other) and snap-dried on a 70-80° C. inverted heating block for 3 seconds. Nucleic acid is then UV crosslinked to the slide (Stratagene, Stratalinker, 65 mJ—set display to "650" which is 650×100 uJ) or the array is baked at 80 C for two to four hours prior to hybridization. The arrays are placed in a slide rack. An empty slide chamber is prepared and filled with the following solution: 3.0 grams of succinic anhydride (Aldrich) was dissolved in 189 ml of 1-methyl-2-pyrrolidinone (rapid addition of reagent is crucial); immediately after the last flake of succinic anhydride is dissolved, ~21.0 ml of 0.2 M sodium borate is mixed in and the solution is poured into the slide chamber. The slide rack is plunged rapidly and evenly in the slide chamber and vigorously shaken up and down for a few seconds, making sure the slides never leave the solution, and then mixed on an orbital shaker for 15-20 minutes. The slide rack is then gently plunged in 95° C. ddH_20 for 2 minutes, followed by plunging five times in 95% ethanol. The slides are then air dried by allowing excess ethanol to drip onto paper towels. The arrays are stored in the slide box at room temperature until use.

[0312] Numerous methods may be used for attachment of the nucleic acid members of the invention to the substrate (a process referred to as "spotting"). For example, nucleic acids are attached using the techniques of, for example U.S. Pat. No. 5,807,522, which is incorporated herein by reference, for teaching methods of polymer attachment.

[0313] Alternatively, spotting may be carried out using contact printing technology as is known in the art.

Use of a Microarray

[0314] Nucleic acid arrays according to the invention can be used in high throughput techniques that can assay a large number of nucleic acids in a sample comprising one or more target nucleic acid sequences. The arrays of the subject invention find use in a variety of applications, including gene expression analysis, diagnosis of osteoarthritis and prognosis of osteoarthritis, monitoring a patient's response to therapy, drug screening, and the like.

[0315] The arrays are also useful in broad scale expression screening for drug discovery and research, such as the effect of a particular active agent on the expression pattern of genes of the invention, where such information is used to reveal drug efficacy and toxicity, environmental monitoring, disease research and the like.

[0316] Arrays can be made using at least one, more preferably a combination of these sequences, as a means of diag-

nosing osteoarthritis, or for purposes of monitoring efficacy of treatment and of identifying stage specific osteoarthritis.

[0317] The choice of a standard sample would be well understood by a person skilled in the art, and would include a sample complementary to RNA isolated from one or more normal individuals, wherein a normal individual is an individual not suffering from osteoarthritis. In the case of monitoring efficacy of treatment or identifying stage specific osteoarthritis, it would be understood by a person skilled in the art that a control would include samples from persons suffering various degrees of osteoarthritis and/or persons responding to treatment. Standard samples would also include a sample complementary to RNA isolated from chondrocytes, or from blood, or from synovial fluid.

Target Preparation

[0318] The targets for the arrays according to the invention are preferably derived from human cartilage, blood or synovial fluid.

[0319] A target nucleic acid is capable of binding to a nucleic acid probe or nucleic acid member of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation.

[0320] As used herein, a "nucleic acid derived from an mRNA transcript: or a "nucleic acid corresponding to an mRNA" refers to a nucleic acid for which synthesis of the mRNA transcript or a sub-sequence thereof has ultimately served as a template. Thus, a cDNA reverse transcribed from an mRNA, an RNA transcribed from that cDNA, a DNA amplified from the cDNA, an RNA transcribed from the amplified DNA, etc., are all derived from or correspond to the mRNA transcript and detection of such derived or corresponding products is indicative of or proportional to the presence and/or abundance of the original transcript in a sample. Thus, suitable target nucleic acid samples include, but are not limited to, mRNA transcripts of a gene or genes, cDNA reverse transcribed from the mRNA, cRNA transcribed from the cDNA, DNA amplified from a gene or genes, RNA transcribed from amplified DNA, and the like. The nucleic acid targets used herein are preferably derived from human cartilage, blood or synovial fluid. Preferably, the targets are nucleic acids derived from human cartilage, blood or synovial fluid extracts. Nucleic acids can be single- or double-stranded DNA, RNA, or DNA-RNA hybrids synthesized from human cartilage, blood or synovial fluid mRNA extracts using methods known in the art, for example, reverse transcription or PCR.

[0321] In the simplest embodiment, such a nucleic acid target comprises total mRNA or a nucleic acid sample corresponding to mRNA (e.g., cDNA) isolated from cartilage, blood, or synovial fluid samples. In another embodiment, total mRNA is isolated from a given sample using, for example, an acid guanidinium-phenol-chloroform extraction method and polyA+ mRNA is isolated by oligo dT column chromatography or by using (dT)n magnetic beads (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989), or Current Protocols in Molecular Biology, F. Ausubel et al., ed. Greene Publishing and Wiley-Interscience, New York (1987). In a preferred embodiment, total RNA is extracted using TRIzol® reagent (GIBCO/BRL, Invitrogen Life Technologies, Cat. No. 15596). Purity and integrity of RNA is assessed by absorbance at 260/280 nm and agarose gel electrophoresis followed by inspection under ultraviolet light.

[0322] In some embodiments, it is desirable to amplify the target nucleic acid sample prior to hybridization, for example, when synovial fluid is used. One of skill in the art will appreciate that whatever amplification method is used, if a quantitative result is desired, care must be taken to use a method that maintains or controls for the relative frequencies of the amplified nucleic acids. Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. The high density array may then include probes specific to the internal standard for quantification of the amplified nucleic acid. Detailed protocols for quantitative PCR are provided in *PCR Protocols, A Guide to Methods and Applications,* Innis et al., Academic Press, Inc. N.Y., (1990).

[0323] Other suitable amplification methods include, but are not limited to polymerase chain reaction (PCR) (Innis, et al., *PCR Protocols. A Guide to Methods and Application.* Academic Press, Inc. San Diego, (1990)), ligase chain reaction (LCR) (see Wu and Wallace, 1989, *Genomics,* 4:560; Landegren, et al., 1988, *Science,* 241:1077 and Barringer, et al., 1990, *Gene,* 89:117, transcription amplification (Kwoh, et al., 1989, *Proc. Natl. Acad. Sci. USA,* 86: 1173), and self-sustained sequence replication (Guatelli, et al., 1990, *Proc. Nat. Acad. Sci. USA,* 87: 1874).

[0324] In a particularly preferred embodiment, the target nucleic acid sample mRNA is reverse transcribed with a reverse transcriptase and a primer consisting of oligo dT and a sequence encoding the phage T7 promoter to provide single-stranded DNA template. The second DNA strand is polymerized using a DNA polymerase. After synthesis of double-stranded cDNA, T7 RNA polymerase is added and RNA is transcribed from the cDNA template. Successive rounds of transcription from each single cDNA template results in amplified RNA. Methods of in vitro transcription are well known to those of skill in the art (see, e.g., Sambrook, supra.) and this particular method is described in detail by Van Gelder, et al., 1990, *Proc. Natl. Acad Sci. USA,* 87: 1663-1667 who demonstrate that in vitro amplification according to this method preserves the relative frequencies of the various RNA transcripts. Moreover, Eberwine et al. *Proc. Natl. Acad. Sci. USA,* 89: 3010-3014 provide a protocol that uses two rounds of amplification via in vitro transcription to achieve greater than 10^6 fold amplification of the original starting material thereby permitting expression monitoring even where biological samples are limited.

Labeling of Target or Nucleic Acid Probe

[0325] Either the target or the probe can be labeled.

[0326] Any analytically detectable marker that is attached to or incorporated into a molecule may be used in the invention. An analytically detectable marker refers to any molecule, moiety or atom which is analytically detected and quantified.

[0327] Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., Dynabeads™), fluorescent

dyes (e.g., fluorescein, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ^{3}H, ^{125}I, 35S, ^{14}C, or ^{32}P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, the entireties of which are incorporated by reference herein.

[0328] Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

[0329] The labels may be incorporated by any of a number of means well known to those of skill in the art. However, in a preferred embodiment, the label is simultaneously incorporated during the amplification step in the preparation of the sample nucleic acids. Thus, for example, polymerase chain reaction (PCR) with labeled primers or labeled nucleotides will provide a labeled amplification product. In a preferred embodiment, transcription amplification, as described above, using a labeled nucleotide (e.g. fluorescein-labeled UTP and/or CTP) incorporates a label into the transcribed nucleic acids.

[0330] Alternatively, a label may be added directly to the original nucleic acid sample (e.g., mRNA, polyA mRNA, cDNA, etc.) or to the amplification product after the amplification is completed. Means of attaching labels to nucleic acids are well known to those of skill in the art and include, for example, nick translation or end-labeling (e.g. with a labeled RNA) by kinasing of the nucleic acid and subsequent attachment (ligation) of a nucleic acid linker joining the sample nucleic acid to a label (e.g., a fluorophore).

[0331] In a preferred embodiment, the fluorescent modifications are by cyanine dyes e.g. Cy-3/Cy-5 dUTP, Cy-3/Cy-5 dCTP (Amersham Pharmacia) or alexa dyes (Khan, et al., 1998, *Cancer Res.* 58:5009-5013).

[0332] In a preferred embodiment, the two target samples used for comparison are labeled with different fluorescent dyes which produce distinguishable detection signals, for example, targets made from normal cartilage are labeled with Cy5 and targets made from mild osteoarthritis cartilage are labeled with Cy3. The differently labeled target samples are hybridized to the same microarray simultaneously. In a preferred embodiment, the labeled targets are purified using methods known in the art, e.g., by ethanol purification or column purification.

[0333] In a preferred embodiment, the target will include one or more control molecules which hybridize to control probes on the microarray to normalize signals generated from the microarray. Preferably, labeled normalization targets are nucleic acid sequences that are perfectly complementary to control oligonucleotides that are spotted onto the microarray as described above. The signals obtained from the normalization controls after hybridization provide a control for variations in hybridization conditions, label intensity, "reading" efficiency and other factors that may cause the signal of a perfect hybridization to vary between arrays. In a preferred embodiment, signals (e.g., fluorescence intensity) read from all other probes in the array are divided by the signal (e.g., fluorescence intensity) from the control probes, thereby normalizing the measurements.

[0334] Preferred normalization targets are selected to reflect the average length of the other targets present in the sample, however, they are selected to cover a range of lengths. The normalization control(s) also can be selected to reflect the (average) base composition of the other probes in the array, however, in a preferred embodiment, only one or a few normalization probes are used and they are selected such that they hybridize well (i.e., have no secondary structure and do not self hybridize) and do not match any target molecules.

[0335] Normalization probes are localized at any position in the array or at multiple positions throughout the array to control for spatial variation in hybridization efficiency. In a preferred embodiment, normalization controls are located at the corners or edges of the array as well as in the middle.

Hybridization Conditions

[0336] Nucleic acid hybridization involves providing a denatured probe or target nucleic acid member and target nucleic acid under conditions where the probe or target nucleic acid member and its complementary target can form stable hybrid duplexes through complementary base pairing. The nucleic acids that do not form hybrid duplexes are then washed away leaving the hybridized nucleic acids to be detected, typically through detection of an attached detectable label. It is generally recognized that nucleic acids are denatured by increasing the temperature or decreasing the salt concentration of the buffer containing the nucleic acids. Under low stringency conditions (e.g., low temperature and/or high salt) hybrid duplexes (e.g., DNA:DNA, RNA:RNA, or RNA:DNA) will form even where the annealed sequences are not perfectly complementary. Thus specificity of hybridization is reduced at lower stringency. Conversely, at higher stringency (e.g., higher temperature or lower salt) successful hybridization requires fewer mismatches.

[0337] The invention provides for hybridization conditions comprising the Dig hybridization mix (Boehringer); or formamide-based hybridization solutions, for example as described in Ausubel et al., supra and Sambrook et al. supra.

[0338] Methods of optimizing hybridization conditions are well known to those of skill in the art (see, e.g., *Laboratory Techniques in Biochemistry and Molecular Biology,* Vol. 24: *Hybridization With Nucleic acid Probes,* P. Tijssen, ed. Elsevier, N.Y., (1993)).

[0339] Following hybridization, non-hybridized labeled or unlabeled nucleic acid is removed from the support surface, conveniently by washing, thereby generating a pattern of hybridized target nucleic acid on the substrate surface. A variety of wash solutions are known to those of skill in the art and may be used. The resultant hybridization patterns of labeled, hybridized oligonucleotides and/or nucleic acids may be visualized or detected in a variety of ways, with the particular manner of detection being chosen based on the particular label of the test nucleic acid, where representative detection means include scintillation counting, autoradiography, fluorescence measurement, calorimetric measurement, light emission measurement and the like.

Image Acquisition and Data Analysis

[0340] Following hybridization and any washing step(s) and/or subsequent treatments, as described above, the result-

ant hybridization pattern is detected. In detecting or visualizing the hybridization pattern, the intensity or signal value of the label will be not only be detected but quantified, by which is meant that the signal from each spot of the hybridization will be measured and compared to a unit value corresponding to the signal emitted by a known number of end labeled target nucleic acids to obtain a count or absolute value of the copy number of each end-labeled target that is hybridized to a particular spot on the array in the hybridization pattern.

[0341] Methods for analyzing the data collected from hybridization to arrays are well known in the art. For example, where detection of hybridization involves a fluorescent label, data analysis can include the steps of determining fluorescent intensity as a function of substrate position from the data collected, removing outliers, i.e., data deviating from a predetermined statistical distribution, and calculating the relative binding affinity of the test nucleic acids from the remaining data. The resulting data is displayed as an image with the intensity in each region varying according to the binding affinity between associated oligonucleotides and/or nucleic acids and the test nucleic acids.

[0342] The following detection protocol is used for the simultaneous analysis of two cartilage samples to be compared, where each sample is labeled with a different fluorescent dye.

[0343] Each element of the microarray is scanned for the first fluorescent color. The intensity of the fluorescence at each array element is proportional to the expression level of that gene in the sample.

[0344] The scanning operation is repeated for the second fluorescent label. The ratio of the two fluorescent intensities provides a highly accurate and quantitative measurement of the relative gene expression level in the two tissue samples.

[0345] In a preferred embodiment, fluorescence intensities of immobilized target nucleic acid sequences were determined from images taken with a custom confocal microscope equipped with laser excitation sources and interference filters appropriate for the Cy3 and Cy5 fluors. Separate scans were taken for each fluor at a resolution of 225 μm^2 per pixel and 65,536 gray levels. Image segmentation to identify areas of hybridization, normalization of the intensities between the two fluor images, and calculation of the normalized mean fluorescent values at each target are as described (Khan, et al., 1998, *Cancer Res.* 58:5009-5013. Chen, et al., 1997, *Biomed. Optics* 2:364-374). Normalization between the images is used to adjust for the different efficiencies in labeling and detection with the two different fluors. This is achieved by equilibrating to a value of one the signal intensity ratio of a set of internal control genes spotted on the array.

[0346] In another preferred embodiment, the array is scanned in the Cy 3 and Cy5 channels and stored as separate 16-bit TIFF images. The images are incorporated and analysed using software which includes a gridding process to capture the hybridization intensity data from each spot on the array. The fluorescence intensity and background-subtracted hybridization intensity of each spot is collected and a ratio of measured mean intensities of Cy5 to Cy3 is calculated. A linear regression approach is used for normalization and assumes that a scatter plot of the measured Cy5 versus Cy3 intensities should have a slope of one. The average of the ratios is calculated and used to rescale the data and adjust the slope to one. A ratio of expression not equal to 1 is used as an indication of differential gene expression.

[0347] In a particularly preferred embodiment, where it is desired to quantify the transcription level (and thereby expression) of one or more nucleic acid sequences in a sample, the target nucleic acid sample is one in which the concentration of the mRNA transcript(s) of the gene or genes, or the concentration of the nucleic acids derived from the mRNA transcript(s), is proportional to the transcription level (and therefore expression level) of that gene. Similarly, it is preferred that the hybridization signal intensity be proportional to the amount of hybridized nucleic acid. While it is preferred that the proportionality be relatively strict (e.g., a doubling in transcription rate results in a doubling in mRNA transcript in the sample nucleic acid pool and a doubling in hybridization signal), one of skill will appreciate that the proportionality can be more relaxed and even non-linear and still provide meaningful results. Thus, for example, an assay where a 5 fold difference in concentration of the target mRNA results in a 3- to 6-fold difference in hybridization intensity is sufficient for most purposes. Where more precise quantification is required, appropriate controls are run to correct for variations introduced in sample preparation and hybridization as described herein. In addition, serial dilutions of "standard" target mRNAs are used to prepare calibration curves according to methods well known to those of skill in the art. Of course, where simple detection of the presence or absence of a transcript is desired, no elaborate control or calibration is required.

[0348] For example, if an nucleic acid member on an array is not labeled after hybridization, this indicates that the gene comprising that nucleic acid member is not expressed in either sample. If a nucleic acid member is labeled with a single color, it indicates that a labeled gene was expressed only in one sample. The labeling of a nucleic acid member comprising an array with both colors indicates that the gene was expressed in both samples. Even genes expressed once per cell are detected (1 part in 100,000 sensitivity). A difference in expression intensity in the two samples being compared is indicative of differential expression, the ratio of the intensity in the two samples being not equal to 1.0, preferably less than 0.7 or greater than 1.2, more preferably less than 0.5 or greater than 1.5.

RT-PCR

[0349] In aspect of the invention, the level of the expression of the RNA products of the biomarkers of the invention can be measured by amplifying the RNA products of the biomarkers from a sample using reverse transcription (RT) in combination with the polymerase chain reaction (PCR). In accordance with one embodiment of the invention, the RT can be quantitative as would be understood to a person skilled in the art.

[0350] Total RNA, or mRNA from a sample is used as a template and a primer specific to the transcribed portion of a biomarker of the invention is used to initiate reverse transcription. Methods of reverse transcribing RNA into cDNA are well known and described in Sambrook et al., 1989, supra. Primer design can be accomplished utilizing commercially available software (e.g., Primer Designer 1.0, Scientific Software etc.). The product of the reverse transcription is subsequently used as a template for PCR.

[0351] PCR provides a method for rapidly amplifying a particular nucleic acid sequence by using multiple cycles of DNA replication catalyzed by a thermostable, DNA-dependent DNA polymerase to amplify the target sequence of interest. PCR requires the presence of a nucleic acid to be ampli-

fied, two single-stranded oligonucleotide primers flanking the sequence to be amplified, a DNA polymerase, deoxyribonucleoside triphosphates, a buffer and salts.

[0352] The method of PCR is well known in the art. PCR, is performed as described in Mullis and Faloona, 1987, Methods Enzymol., 155: 335, which is incorporated herein by reference. PCR is performed using template DNA (at least 1 fg; more usefully, 1-1000 ng) and at least 25 pmol of oligonucleotide primers. A typical reaction mixture includes: 2 μl of DNA, 25 pmol of oligonucleotide primer, 2.5 μl of 10H PCR buffer 1 (Perkin-Elmer, Foster City, Calif.), 0.4 μl of 1.25 μM dNTP, 0.15 μl (or 2.5 units) of Taq DNA polymerase (Perkin Elmer, Foster City, Calif.) and deionized water to a total volume of 25 μl. Mineral oil is overlaid and the PCR is performed using a programmable thermal cycler.

[0353] The length and temperature of each step of a PCR cycle, as well as the number of cycles, are adjusted according to the stringency requirements in effect. Annealing temperature and timing are determined both by the efficiency with which a primer is expected to anneal to a template and the degree of mismatch that is to be tolerated. The ability to optimize the stringency of primer annealing conditions is well within the knowledge of one of moderate skill in the art. An annealing temperature of between 30° C. and 72° C. is used. Initial denaturation of the template molecules normally occurs at between 92° C. and 99° C. for 4 minutes, followed by 20-40 cycles consisting of denaturation (94-99° C. for 15 seconds to 1 minute), annealing (temperature determined as discussed above; 1-2 minutes), and extension (72° C. for 1 minute). The final extension step is generally carried out for 4 minutes at 72° C., and may be followed by an indefinite (0-24 hour) step at 4° C.

[0354] QRT-PCR, which is quantitative in nature, can also be performed to provide a quantitative measure of gene expression levels. In QRT-PCR reverse transcription and PCR can be performed in two steps, or reverse transcription combined with PCR can be performed concurrently. One of these techniques, for which there are commercially available kits such as Taqman (Perkin Elmer, Foster City, Calif.), is performed with a transcript-specific antisense probe. This probe is specific for the PCR product (e.g. a nucleic acid fragment derived from a gene) and is prepared with a quencher and fluorescent reporter probe complexed to the 5' end of the oligonucleotide. Different fluorescent markers are attached to different reporters, allowing for measurement of two products in one reaction. When Taq DNA polymerase is activated, it cleaves off the fluorescent reporters of the probe bound to the template by virtue of its 5'-to-3' exonuclease activity. In the absence of the quenchers, the reporters now fluoresce. The color change in the reporters is proportional to the amount of each specific product and is measured by a fluorometer; therefore, the amount of each color is measured and the PCR product is quantified. The PCR reactions are performed in 96 well plates so that samples derived from many individuals are processed and measured simultaneously. The Taqman system has the additional advantage of not requiring gel electrophoresis and allows for quantification when used with a standard curve.

[0355] A second technique useful for detecting PCR products quantitatively without is to use an intercolating dye such as the commercially available QuantiTect SYBR Green PCR (Qiagen, Valencia Calif.). RT-PCR is performed using SYBR green as a fluorescent label which is incorporated into the PCR product during the PCR stage and produces a flourescense proportional to the amount of PCR product.

[0356] Both Taqman and QuantiTect SYBR systems can be used subsequent to reverse transcription of RNA. Reverse transcription can either be performed in the same reaction mixture as the PCR step (one-step protocol) or reverse transcription can be performed first prior to amplification utilizing PCR (two-step protocol).

[0357] Additionally, other systems to quantitatively measure mRNA expression products are known including Molecular Beacons® which uses a probe having a fluorescent molecule and a quencher molecule, the probe capable of forming a hairpin structure such that when in the hairpin form, the fluorescence molecule is quenched, and when hybridized the flourescense increases giving a quantitative measurement of gene expression.

[0358] Additional techniques to quantitatively measure RNA expression include, but are not limited to, polymerase chain reaction, ligase chain reaction, Qbeta replicase (see, e.g., International Application No. PCT/US87/00880), isothermal amplification method (see, e.g., Walker et al. (1992) PNAS 89:382-396), strand displacement amplification (SDA), repair chain reaction, Asymmetric Quantitative PCR (see, e.g., U.S. Publication No. US200330134307A1) and the multiplex microsphere bead assay described in Fuja et al., 2004, Journal of Biotechnology 108:193-205.

[0359] The level of gene expression can be measured by amplifying RNA from a sample using transcription based amplification systems (TAS), including nucleic acid sequence amplification (NASBA) and 3SR. See, e.g., Kwoh et al (1989) PNAS USA 86:1173; International Publication No. WO 88/10315; and U.S. Pat. No. 6,329,179. In NASBA, the nucleic acids may be prepared for amplification using conventional phenol/chloroform in extraction, heat denaturation, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer that has target specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target specific primer, followed by polymerization. The double-stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNA's are reverse transcribed into double stranded DNA, and transcribed once with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target specific sequences.

[0360] Several techniques may be used to separate amplification products. For example, amplification products may be separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using conventional methods. See Sambrook et al., 1989. Several techniques for detecting PCR products quantitatively without electrophoresis may also be used according to the invention (see for example *PCR Protocols, A Guide to Methods and Applications,* Innis et al., Academic Press, Inc. N.Y., (1990)). For example, chromatographic techniques may be employed to effect separation. There are many kinds of chromatography which may be used in the present invention: adsorption, partition, ion-exchange and molecular sieve, HPLC, and many specialized techniques for using them including column, paper, thin-layer and gas chromatography (Freifelder, Physical Biochemistry Applica-

tions to Biochemistry and Molecular Biology, 2nd ed., Wm. Freeman and Co., New York, N.Y., 1982).

[0361] Another example of a separation methodology is done by covalently labeling the oligonucleotide primers used in a PCR reaction with various types of small molecule ligands. In one such separation, a different ligand is present on each oligonucleotide. A molecule, perhaps an antibody or avidin if the ligand is biotin, that specifically binds to one of the ligands is used to coat the surface of a plate such as a 96 well ELISA plate. Upon application of the PCR reactions to the surface of such a prepared plate, the PCR products are bound with specificity to the surface. After washing the plate to remove unbound reagents, a solution containing a second molecule that binds to the first ligand is added. This second molecule is linked to some kind of reporter system. The second molecule only binds to the plate if a PCR product has been produced whereby both oligonucleotide primers are incorporated into the final PCR products. The amount of the PCR product is then detected and quantified in a commercial plate reader much as ELISA reactions are detected and quantified. An ELISA-like system such as the one described here has been developed by the Raggio Italgene company under the C-Track trade name.

[0362] Amplification products must be visualized in order to confirm amplification of the nucleic acid sequences of interest. One typical visualization method involves staining of a gel with ethidium bromide and visualization under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the amplification products may then be exposed to x-ray film or visualized under the appropriate stimulating spectra, following separation.

[0363] In one embodiment, visualization is achieved indirectly. Following separation of amplification products, a labeled, nucleic acid probe is brought into contact with the amplified nucleic acid sequence of interest. The probe preferably is conjugated to a chromophore but may be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, where the other member of the binding pair carries a detectable moiety.

[0364] In another embodiment, detection is by Southern blotting and hybridization with a labeled probe. The techniques involved in Southern blotting are well known to those of skill in the art and may be found in many standard books on molecular protocols. See Sambrook et al., 1989, supra. Briefly, amplification products are separated by gel electrophoresis. The gel is then contacted with a membrane, such as nitrocellulose, permitting transfer of the nucleic acid and non-covalent binding. Subsequently, the membrane is incubated with a chromophore-conjugated probe that is capable of hybridizing with a target amplification product. Detection is by exposure of the membrane to x-ray film or ion-emitting detection devices.

[0365] One example of the foregoing is described in U.S. Pat. No. 5,279,721, incorporated by reference herein, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.

Nuclease Protection Assays

[0366] In another embodiment of the invention, Nuclease protection assays (including both ribonuclease protection assays and S1 nuclease assays) can be used to detect and quantitate the RNA products of the biomarkers of the invention. In nuclease protection assays, an antisense probe (labeled with, e.g., radiolabeled or nonisotopic) hybridizes in solution to an RNA sample. Following hybridization, single-stranded, unhybridized probe and RNA are degraded by nucleases. An acrylamide gel is used to separate the remaining protected fragments. Typically, solution hybridization is more efficient than membrane-based hybridization, and it can accommodate up to 100 μg of sample RNA, compared with the 20-30 μg maximum of blot hybridizations.

[0367] The ribonuclease protection assay, which is the most common type of nuclease protection assay, requires the use of RNA probes. Oligonucleotides and other single-stranded DNA probes can only be used in assays containing S1 nuclease. The single-stranded, antisense probe must typically be completely homologous to target RNA to prevent cleavage of the probe:target hybrid by nuclease.

Northern Blots

[0368] A standard Northern blot assay can also be used to ascertain an RNA transcript size, identify alternatively spliced RNA transcripts, and the relative amounts of RNA products of the biomarker of the invention, in accordance with conventional Northern hybridization techniques known to those persons of ordinary skill in the art. In Northern blots, RNA samples are first separated by size via electrophoresis in an agarose gel under denaturing conditions. The RNA is then transferred to a membrane, crosslinked and hybridized with a labeled probe. Nonisotopic or high specific activity radiolabeled probes can be used including random-primed, nick-translated, or PCR-generated DNA probes, in vitro transcribed RNA probes, and oligonucleotides. Additionally, sequences with only partial homology (e.g., cDNA from a different species or genomic DNA fragments that might contain an exon) may be used as probes. The labeled probe, e.g., a radiolabelled cDNA, either containing the full-length, single stranded DNA or a fragment of that DNA sequence may be at least 20, at least 30, at least 50, or at least 100 consecutive nucleotides in length. The probe can be labeled by any of the many different methods known to those skilled in this art. The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals that fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, but are not limited to, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. Non-limiting examples of isotopes include ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re. Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Any enzymes known to one of skill in the art can be utilized. Examples of such enzymes include, but are not limited to, peroxidase, beta-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Pat. Nos.

3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

5.18 Techniques to Measure the Protein Products of the Biomarkers of the Invention

Protein Products

[0369] Standard techniques can also be utilized for determining the amount of the protein or proteins of interest present in a sample. For example, standard techniques can be employed using, e.g., immunoassays such as, for example, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), immunocytochemistry, and the like to determine the amount of the protein or proteins of interest present in a sample. A preferred agent for detecting a protein of interest is an antibody capable of binding to a protein of interest, preferably an antibody with a detectable label.

[0370] For such detection methods, protein from the sample to be analyzed can easily be isolated using techniques which are well known to those of skill in the art. Protein isolation methods can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988)).

[0371] Preferred methods for the detection of the protein or proteins of interest involve their detection via interaction with a protein-specific antibody. For example, antibodies directed a protein of interest can be utilized as described herein. Antibodies can be generated utilizing standard techniques well known to those of skill in the art. See, e.g., Section 5.5.1 of this application and Section 5.2 of U.S. Publication No. 20040018200 for a more detailed discussion of such antibody generation techniques, which is incorporated herein by reference. Briefly, such antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or an antibody fragment (e.g., Fab or $F(ab')_2$) can, for example, be used. Preferably, the antibody is a human or humanized antibody.

[0372] For example, antibodies, or fragments of antibodies, specific for a protein of interest can be used to quantitatively or qualitatively detect the presence of the protein. This can be accomplished, for example, by immunofluorescence techniques. Antibodies (or fragments thereof) can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of a protein of interest. In situ detection can be accomplished by removing a histological specimen (e.g., a biopsy specimen) from a patient, and applying thereto a labeled antibody thereto that is directed to a protein. The antibody (or fragment) is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the protein of interest, but also its distribution, its presence in cells (e.g., chondrocytes and lymphocytes) within the sample. A wide variety of well-known histological methods (such as staining procedures) can be utilized in order to achieve such in situ detection.

[0373] Immunoassays for a protein of interest typically comprise incubating a biological sample of a detectably labeled antibody capable of identifying a protein of interest, and detecting the bound antibody by any of a number of techniques well-known in the art. As discussed in more detail, below, the term "labeled" can refer to direct labeling of the antibody via, e.g., coupling (i.e., physically linking) a detectable substance to the antibody, and can also refer to indirect labeling of the antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody.

[0374] For example, the biological sample can be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific antibody. The solid phase support can then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on solid support can then be detected by conventional means.

[0375] By "solid phase support or carrier" in the context of proteinaceous agents is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material can have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface can be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0376] One of the ways in which a specific antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, Md.); Voller, A. et al., 1978, J. Clin. Pathol. 31:507-520; Butler, J. E., 1981, Meth. Enzymol. 73:482-523; Maggio, E. (ed.), 1980, *Enzyme Immunoassay,* CRC Press, Boca Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, *Enzyme Immunoassay,* Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0377] Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is

possible to detect a protein of interest through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope (e.g., ^{125}I, ^{131}I, ^{35}S or ^{3}H) can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

[0378] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

[0379] The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0380] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0381] Likewise, a bioluminescent compound can be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Protein Arrays

[0382] Polypeptides which specifically and/or selectively bind to the protein products of the biomarkers of the invention can be immobilized on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such as isolated cells, blood, synovial fluid, sera, biopsies, and the like) for the presence of the polypeptides protein products of the biomarkers of the invention. The protein array can also include antibodies as well as other ligands, e.g., that bind to the polypeptides encoded by the biomarkers of the invention.

[0383] Methods of producing polypeptide arrays are described, e.g., in De Wildt et al., 2000, Nature Biotech. 18:989-994; Lueking et al., 1999, Anal. Biochem. 270:103-111; Ge, 2000, Nuc. Acids Res. 28:e3 ; MacBeath and Schreiber, 2000, Science 289:1760-1763; International Publication Nos. WO 01/40803 and WO 99/51773A1; and U.S. Pat. No. 6,406,921. Polypeptides for the array can be spotted at high speed, e.g., using commercially available robotic apparatus, e.g., from Genetic MicroSystems and Affymetrix (Santa Clara, Calif., USA) or BioRobotics (Cambridge, UK). The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose, or another polymer.

[0384] For example, the array can be an array of antibodies, e.g., as described in De Wildt, supra. Cells that produce the polypeptide ligands can be grown on a filter in an arrayed format. Polypeptide production is induced, and the expressed antibodies are immobilized to the filter at the location of the cell. Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer database.

[0385] In one embodiment the array is an array of protein products of the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. In one aspect, the invention provides for antibodies that are bound to an array which selectively bind to the protein products of the biomarkers of the invention.

5.19 Protein Production

[0386] Standard recombinant nucleic acid methods can be used to express a polypeptide or antibody of the invention (e.g., a protein product of a biomarker of the invention). Generally, a nucleic acid sequence encoding the polypeptide is cloned into a nucleic acid expression vector. Of course, if the protein includes multiple polypeptide chains, each chain must be cloned into an expression vector, e.g., the same or different vectors, that are expressed in the same or different cells. If the protein is sufficiently small, i.e., the protein is a peptide of less than 50 amino acids, the protein can be synthesized using automated organic synthetic methods. Polypeptides comprising the 5' region, 3' region or internal coding region of a biomarker of the invention, are expressed from nucleic acid expression vectors containing only those nucleotide sequences corresponding to the 5' region, 3' region or internal coding region of a biomarker of the invention. Methods for producing antibodies directed to protein products of a biomarker of the invention, or polypeptides encoded by the 5' region, 3' region or internal coding regions of a biomarker of the invention.

[0387] The expression vector for expressing the polypeptide can include, in addition to the segment encoding the polypeptide or fragment thereof, regulatory sequences, including for example, a promoter, operably linked to the nucleic acid(s) of interest. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). One preferred class of preferred libraries is the display library, which is described below.

[0388] Methods well known to those skilled in the art can be used to construct vectors containing a polynucleotide of the invention and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Sambrook & Russell, *Molecular Cloning: A Laboratory Manual,* 3rd Edition, Cold Spring Harbor Laboratory, N.Y. (2001) and Ausubel et al., *Current Protocols in Molecular Biology* (Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Promoter regions can be selected from any desired gene using CAT (chloramphenicol

transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lad, lacZ, T3, T7, gpt, lambda P, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, mouse metallothionein-I, and various art-known tissue specific promoters. In specific embodiments, the promoter is an inducible promoter. In other embodiments, the promoter is a constitutive promoter. In yet other embodiments, the promoter is a tissue-specific promoter.

[0389] Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* auxotrophic markers (such as URA3, LEU2, HIS3, and TRP1 genes), and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The polynucleotide of the invention is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, a nucleic acid of the invention can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Useful expression-vectors for bacteria are constructed by inserting a polynucleotide of the invention together with suitable translation initiation and termination signals, optionally in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas, Streptomyces,* and *Staphylococcus,* although others may also be employed as a matter of choice.

[0390] As a representative but nonlimiting example, useful expression vectors for bacteria can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega, Madison, Wis., USA).

[0391] The present invention provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention also provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

[0392] The present invention further provides host cells containing the vectors of the present invention, wherein the nucleic acid has been introduced into the host cell using known transformation, transfection or infection methods. The host cell can be a eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected, for example, by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

[0393] Any host/vector system can be used to express one or more of the genes listed in Table 2 or splice variants. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., in *Molecular Cloning: A Laboratory Manual,* Second Edition, Cold Spring Harbor, N.Y. (1989), the disclosure of which is incorporated herein by reference in its entirety. The most preferred host cells are those which do not normally express the particular polypeptide or which expresses the polypeptide at low natural level.

[0394] In a specific embodiment, the host cells are engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting, including polyadenylation signals. mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

[0395] The host of the present invention may also be a yeast or other fungi. In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Ausubel et al. (eds), *Current Protocols in Molecular Biology,* Vol. 2, Greene Publish. Assoc. & Wiley Interscience, Ch. 13 (1988); Grant et al., 1987, "Expression and Secretion Vectors for Yeast", Methods Enzymol. 153:516-544; Glover, *DNA Cloning,* Vol. II, IRL Press, Wash., D.C., Ch. 3 (1986); Bitter, 1987, "Heterologous Gene Expression in Yeast", Methods Enzymol. 152:673-684; and Strathern et al. (eds), *The Molecular Biology of the Yeast Saccharomyces,* Cold Spring Harbor Press, Vols. I and II (1982).

[0396] Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli,* enterobacteriaceae such as *Serratia marescans,* bacilli such as *Bacillus subtilis, Salmonella typhimurium,* pseudomonads or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

[0397] Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the monkey COS cells such as COS-7 lines of monkey kidney fibroblasts, described by Gluzman, 1981, Cell 23:175 (1981), Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, C127, 3T3, or Jurkat cells, and other cell lines capable of expressing a compatible vector. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome-binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences.

[0398] Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Recombinant polypeptides produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. In some embodiments, the template nucleic acid also encodes a polypeptide tag, e.g., penta- or hexa-histidine.

[0399] Recombinant proteins can be isolated using an technique well-known in the art. Scopes (*Protein Purification: Principles and Practice,* Springer-Verlag, New York (1994)), for example, provides a number of general methods for purifying recombinant (and non-recombinant) proteins. The methods include, e.g., ion-exchange chromatography, size-exclusion chromatography, affinity chromatography, selective precipitation, dialysis, and hydrophobic interaction chromatography.

[0400] Variations, modifications, and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention.

[0401] In order that the invention described herein may be more fully understood, the following example is set forth. It should be understood that this example is for illustrative purposes only and are not to be construed as limiting this invention in any manner.

5.20 Methods for Identifying Compounds for Use in the Prevention, Treatment, Management or Amelioration Osteoarthritis or a Symptom Thereof

5.20.1 Methods for Identifying Compounds that Modulate the Expression or Activity of a Biomarker

[0402] The present invention provides methods of identifying compounds that bind to the products of the biomarkers of the invention. The present invention also provides methods for identifying compounds that modulate the expression and/or activity of the products of the biomarkers of the invention. The compounds identified via such methods are useful for the development of one or more animal models to study osteoarthritis. Further, the compounds identified via such methods are useful as lead compounds in the development of prophylactic and therapeutic compositions for prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof. Such methods are particularly useful in that the effort and great expense involved in testing potential prophylactics and therapeutics in vivo is efficiently focused on those compounds identified via the in vitro and ex vivo methods described herein.

[0403] The present invention provides a method for identifying a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, said method comprising: (a) contacting a cell expressing a protein product of one or more biomarkers of the invention or a fragment thereof, or a RNA product of one or more biomarkers of the invention or a fragment thereof with a test compound; and (b) determining the ability of the test compound to bind to the protein product, protein fragment, RNA product, or RNA portion so that if a compound binds to the protein product, protein fragment, RNA product, RNA portion, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. The cell, for example, can be a yeast cell or a cell of mammalian origin. Determining the ability of the test compound to bind to the protein product, protein fragment, RNA product, or RNA portion can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the protein product, protein fragment, RNA product, or RNA portion can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In a specific embodiment, the assay comprises contacting a cell which expresses a protein product of one or more biomarkers of the invention or a fragment thereof, or a RNA product of one or more biomarkers of the invention or a fragment thereof, with a known compound which binds the protein product, protein fragment, RNA product, or RNA portion to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the protein product, protein fragment, RNA product, or RNA portion, wherein determining the ability of the test compound to interact with the protein product, protein fragment, RNA product, or RNA portion comprises determining the ability of the test compound to preferentially bind to the protein product, protein fragment, RNA product, or RNA portion as compared to the known compound.

[0404] The present invention provides a method for identifying a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, said method comprising: (a) contacting a protein product of one or more biomarkers of the invention or a fragment thereof, or a RNA product of one or more biomarkers of the invention or a portion thereof with a test compound; and (b) determining the ability of the test compound to bind to the protein product, protein fragment, RNA product, or RNA portion so that if a compound binds to the protein product, protein fragment, RNA product, or RNA portion, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. Binding of the test compound to the protein product or protein fragment can be determined either directly or indirectly. In a specific embodiment, the assay includes contacting a protein product of one or more biomarkers of the invention or a

fragment thereof, or a RNA product of one or more biomarkers of the invention or a portion thereof with a known compound which binds the protein product, protein fragment, RNA product, or RNA portion to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the protein product, protein fragment, RNA product, or RNA portion, wherein determining the ability of the test compound to interact with the protein product, protein fragment, RNA product, or RNA portion comprises determining the ability of the test compound to preferentially bind to the protein product, protein fragment, RNA product, or RNA portion as compared to the known compound. Techniques well known in the art can be used to determine the binding between a test compound and a protein product of a biomarker of the invention or a fragment thereof, or a RNA product of a biomarker of the invention or a portion thereof.

[0405] In some embodiments of the above assay methods of the present invention, it may be desirable to immobilize a RNA product of a biomarker of the invention or a portion thereof, or its target molecule to facilitate separation of complexed from uncomplexed forms of the RNA product or RNA portion, the target molecule or both, as well as to accommodate automation of the assay. In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either a protein product of a biomarker of the invention or a fragment thereof, or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a protein product of a biomarker of the invention or a fragment thereof can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase (GST) fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or a protein product of a biomarker of the invention or a fragment thereof, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of binding of a protein product of a biomarker of the invention or a fragment thereof can be determined using standard techniques.

[0406] Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a protein product of a biomarker of the invention or a fragment thereof, or a target molecule can be immobilized utilizing conjugation of biotin and streptavidin. A biotinylated protein product of a biomarker of the invention or a target molecule can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with a protein product of a biomarker of the invention or a fragment thereof can be derivatized to the wells of the plate, and protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with a protein product of a biomarker of the invention, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with a protein product of a biomarker of the invention or a fragment thereof, or target molecule.

[0407] The interaction or binding of a protein product of a biomarker of the invention or a fragment thereof to a test compound can also be determined using such proteins or protein fragments as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and International Publication No. WO 94/10300).

[0408] The present invention provides a method for identifying a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, said method comprising: (a) contacting a cell expressing a protein or RNA product of one or more biomarkers of the invention with a test compound; (b) determining the amount of the protein or RNA product present in (a); and (c) comparing the amount in (a) to that present in a corresponding control cell that has not been contacted with the test compound, so that if the amount of the protein or RNA product is altered relative to the amount in the control; a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. In a specific embodiment, the expression level(s) is altered by 5%, 10%, 15%, 25%, 30%, 40%, 50%, 5 to 25%, 10 to 30%, at least 1 fold, at least 1.5 fold, at least 2 fold, 4 fold, 5 fold, 10 fold, 25 fold, 1 to 10 fold, or 5 to 25 fold relative to the expression level in the control as determined by utilizing an assay described herein (e.g., a microarray or RT-PCR) or an assay well known to one of skill in the art. In alternate embodiments, such a method comprises determining the amount of the protein or RNA product of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, 1 to 5, 1-10, 5-10, 5-25, or 10-40, all or any combination of the biomarkers of the invention present in the cell and comparing the amounts to those present in the control.

[0409] The cells utilized in the cell-based assays described herein can be engineered to express a biomarker of the invention utilizing techniques known in the art. See, e.g., Section III entitled "Recombinant Expression Vectors and Host Cells" of U.S. Pat. No. 6,245,527, which is incorporated herein by reference. Alternatively, cells that endogenously express a biomarker of the invention can be used. For example, chondrocytes may be used.

[0410] In a specific embodiment, chondrocytes are isolated from a "normal" individual, or an individual with mild, moderate, marked or severe osteoarthritis and are incubated in the presence and absence of a test compound for varying amounts of time (i.e., 30 min, 1 hr, 5 hr, 24 hr, 48 hr and 96 hrs). When screening for prophylactic or therapeutic agents, a clone of the full sequence of a biomarker of the invention or functional portion thereof is used to transfect chondrocytes. The transfected chondrocytes are cultured for varying amounts of time

(i.e., 1, 2, 3, 5, 7, 10, or 14 days) in the presence or absence of test compound. Following incubation, target nucleic acid samples are prepared from the chondrocytes and hybridized to a nucleic acid probe corresponding to a nucleic acid sequence which is differentially expressed in a chondrocyte derived from at least any two of the following of: normal, mild osteoarthritic, moderate osteoarthritic and severe osteoarthritic. The nucleic acid probe is labeled, for example, with a radioactive label, according to methods well-known in the art and described herein. Hybridization is carried out by northern blot, for example as described in Ausubel et al., supra or Sambrook et al., supra). The differential hybridization, as defined herein, of the target to the samples on the array from normal relative to RNA from any one of mild osteoarthritic, moderate osteoarthritic, marked osteoarthritic and severe osteoarthritic is indicative of the level of expression of RNA corresponding to a differentially expressed chondrocyte specific nucleic acid sequence. A change in the level of expression of the target sequence as a result of the incubation step in the presence of the test compound, is indicative of a compound that increases or decreases the expression of the corresponding chondrocyte specific nucleic acid sequence.

[0411] The present invention also provides a method for identifying a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, said method comprises: (a) contacting a cell-free extract (e.g., a chondrocyte extract) with a nucleic acid sequence encoding a protein or RNA product of one or more biomarkers of the invention and a test compound; (b) determining the amount of the protein or RNA product present in (a); and (c) comparing the amount(s) in (a) to that present to a corresponding control that has not been contacted with the test compound, so that if the amount of the protein or RNA product is altered relative to the amount in the control, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. In a specific embodiment, the expression level(s) is altered by 5%, 10%, 15%, 25%, 30%, 40%, 50%, 5 to 25%, 10 to 30%, at least 1 fold, at least 1.5 fold, at least 2 fold, 4 fold, 5 fold, 10 fold, 25 fold, 1 to 10 fold, or 5 to 25 fold relative to the expression level in the control sample determined by utilizing an assay described herein (e.g., a microarray or RT-PCR) or an assay well known to one of skill in the art. In alternate embodiments, such a method comprises determining the amount of a protein or RNA product of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, 1 to 5, 1-10, 5-10, 5-25, or 10-40, all or any combination of the biomarkers of the invention present in the extract and comparing the amounts to those present in the control.

[0412] In certain embodiments, the amount of RNA product of a biomarker of the invention is determined, in other embodiments, the amount of protein product of a biomarker of the invention is determined, while in still other embodiments, the amount of RNA and protein product of a biomarker of the invention is determined. Standard methods and compositions for determining the amount of RNA or protein product of a biomarker of the invention can be utilized. Such methods and compositions are described in detail above.

[0413] In specific embodiments, in a screening assay described herein, the amount of protein or RNA product of a biomarker of the invention is determined utilizing kits. Such kits comprise materials and reagents required for measuring the expression of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, or more protein or RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention. In specific embodiments, the kits may further comprise one or more additional reagents employed in the various methods, such as: (1) reagents for purifying RNA from blood, chondrocytes or synovial fluid; (2) primers for generating test nucleic acids; (3) dNTPs and/or rNTPs (either premixed or separate), optionally with one or more uniquely labeled dNTPs and/or rNTPs (e.g., biotinylated or Cy3 or Cy5 tagged dNTPs); (4) post synthesis labeling reagents, such as chemically active derivatives of fluorescent dyes; (5) enzymes, such as reverse transcriptases, DNA polymerases, and the like; (6) various buffer mediums, e.g., hybridization and washing buffers; (7) labeled probe purification reagents and components, like spin columns, etc.; and (8) protein purification reagents; (9) signal generation and detection reagents, e.g., streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like. In particular embodiments, the kits comprise prelabeled quality controlled protein and or RNA transcript (preferably, mRNA) for use as a control.

[0414] In some embodiments, the kits are RT-PCR kits. In other embodiments, the kits are nucleic acid arrays and protein arrays. Such kits according to the subject invention will at least comprise an array having associated protein or nucleic acid members of the invention and packaging means therefore. Alternatively the protein or nucleic acid members of the invention may be prepackaged onto an array.

[0415] In a specific embodiment, kits for measuring a RNA product of a biomarker of the invention comprise materials and reagents that are necessary for measuring the expression of the RNA product. For example, a microarray or RT-PCR kit may be used and contain only those reagents and materials necessary for measuring the levels of RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention. Alternatively, in some embodiments, the kits can comprise materials and reagents that are not limited to those required to measure the levels of RNA products of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. For example, a microarray kit may contain reagents and materials necessary for measuring the levels of RNA products 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention, in addition to reagents and materials necessary for measuring the levels of the RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more genes other than the biomarkers of the invention. In a specific embodiment, a microarray or RT-PCR kit contains reagents and materials necessary for measuring the levels of RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the

biomarkers of the invention, and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 300, 350, 400, 450, or more genes that are not biomarkers of the invention, or 1-10, 1-100, 1-150, 1-200, 1-300, 1-400, 1-500, 1-1000, 25-100, 25-200, 25-300, 25-400, 25-500, 25-1000, 100-150, 100-200, 100-300, 100-400, 100-500, 100-1000 or 500-1000 genes that are not biomarkers of the invention.

[0416] For nucleic acid micoarray kits, the kits generally comprise probes attached to a solid support surface. The probes may be labeled with a detectable label. In a specific embodiment, the probes are specific for the 5' region, the 3' region, the internal coding region, an exon(s), an intron(s), an exon junction(s), or an exon-intron junction(s), of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. The microarray kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay. The kits may also comprise hybridization reagents and/or reagents necessary for detecting a signal produced when a probe hybridizes to a target nucleic acid sequence. Generally, the materials and reagents for the microarray kits are in one or more containers. Each component of the kit is generally in its own a suitable container.

[0417] For RT-PCR kits, the kits generally comprise preselected primers specific for particular RNA products (e.g., an exon(s), an intron(s), an exon junction(s), and an exon-intron junction(s)) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. The RT-PCR kits may also comprise enzymes suitable for reverse transcribing and/or amplifying nucleic acids (e.g., polymerases such as Taq), and deoxynucleotides and buffers needed for the reaction mixture for reverse transcription and amplification. The RT-PCR kits may also comprise probes specific for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. The probes may or may not be labeled with a detectable label (e.g., a fluorescent label). Each component of the RT-PCR kit is generally in its own suitable container. Thus, these kits generally comprise distinct containers suitable for each individual reagent, enzyme, primer and probe. Further, the RT-PCR kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay.

[0418] For antibody based kits, the kit can comprise, for example: (1) a first antibody (which may or may not be attached to a solid support) which binds to protein of interest (e.g., a protein product of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention); and, optionally, (2) a second, different antibody which binds to either the protein, or the first antibody and is conjugated to a detectable label (e.g., a fluorescent label, radioactive isotope or enzyme). The antibody-based kits may also comprise beads for conducting an immunoprecipitation. Each component of the antibody-based kits is generally in its own suitable container. Thus, these kits generally comprise distinct containers suitable for each antibody. Further, the antibody-based kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay.

[0419] Reporter gene-based assays may also be conducted to identify a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In a specific embodiment, the present invention provides a method for identifying a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, said method comprising: (a) contacting a compound with a cell expressing a reporter gene construct comprising a reporter gene operably linked to a regulatory element of a biomarker of the invention (e.g., a promoter/enhancer element); (b) measuring the expression of said reporter gene; and (c) comparing the amount in (a) to that present in a corresponding control cell that has not been contacted with the test compound, so that if the amount of expressed reporter gene is altered relative to the amount in the control cell, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. In accordance with this embodiment, the cell may naturally express the biomarker or be engineered to express the biomarker. In another embodiment, the present invention provides a method for identifying a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, said method comprising: (a) contacting a compound with a cell-free extract and a reporter gene construct comprising a reporter gene operably linked to a regulatory element of a biomarker of the invention (e.g., a promoter/enhancer element); (b) measuring the expression of said reporter gene; and (c) comparing the amount in (a) to that present in a corresponding control that has not been contacted with the test compound, so that if the amount of expressed reporter gene is altered relative to the amount in the control, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified.

[0420] Any reporter gene well-known to one of skill in the art may be used in reporter gene constructs used in accordance with the methods of the invention. Reporter genes refer to a nucleotide sequence encoding a RNA transcript or protein that is readily detectable either by its presence (by, e.g., RT-PCR, Northern blot, Western Blot, ELISA, etc.) or activity. Non-limiting examples of reporter genes are listed in Table 4, infra. Reporter genes may be obtained and the nucleotide sequence of the elements determined by any method well-known to one of skill in the art. The nucleotide sequence of a reporter gene can be obtained, e.g., from the literature or a database such as GenBank. Alternatively, a polynucleotide encoding a reporter gene may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular reporter gene is not available, but the sequence of the reporter gene is known, a nucleic acid encoding the reporter gene may be chemically synthesized or obtained from a suitable source (e.g., a cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the reporter gene) by PCR amplification. Once the nucleotide sequence of a reporter gene is determined, the nucleotide sequence of the reporter gene may be manipulated using methods well-known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate reporter genes having a different

amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

TABLE 4

Reporter Genes and the Properties of the Reporter Gene Products

Reporter Gene	Protein Activity & Measurement
CAT (chloramphenicol acetyltransferase)	Transfers radioactive acetyl groups to chloramphenicol or detection by thin layer chromatography and autoradiography
GAL (beta-galactosidase)	Hydrolyzes colorless galactosides to yield colored products.
GUS (beta-glucuronidase)	Hydrolyzes colorless glucuronides to yield colored products.
LUC (luciferase)	Oxidizes luciferin, emitting photons
GFP (green fluorescent protein)	Fluorescent protein without substrate
SEAP (secreted alkaline phosphatase)	Luminescence reaction with suitable substrates or with substrates that generate chromophores
HRP (horseradish peroxidase)	In the presence of hydrogen oxide, oxidation of 3,3',5,5'-tetramethylbenzidine to form a colored complex
AP (alkaline phosphatase)	Luminescence reaction with suitable substrates or with substrates that generate chromophores

[0421] In accordance with the invention, cells that naturally or normally express one or more, all or any combination of the biomarkers of the invention can be used in the methods described herein. Alternatively, cells can be engineered to express one or more, all or any combination of the biomarkers of the invention, or a reporter gene using techniques well-known in the art and used in the methods described herein. Examples of such techniques include, but are not to, calcium phosphate precipitation (see, e.g., Graham & Van der Eb, 1978, Virol. 52:546), dextran-mediated transfection, calcium phosphate mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the nucleic acid in liposomes, and direct microinjection of the nucleic acid into nuclei.

[0422] In a specific embodiment, the cells used in the methods described herein are chondrocytes, lymphocytes (T or B lymphocytes), monocytes, neutrophils, macrophages, eosinophils, basophils, erythrocytes or platelets. In a preferred embodiment, the cells used in the methods described herein are chondrocytes. In another preferred embodiment, the cells used in the methods described herein are lymphocytes. In another embodiment, the cells used in the methods described herein are immortalized cell lines derived from a source, e.g., a tissue.

[0423] Any cell-free extract that permits the translation, and optionally but preferably, the transcription, of a nucleic acid can be used in accordance with the methods described herein. The cell-free extract may be isolated from cells of any species origin. For example, the cell-free translation extract may be isolated from human cells, cultured mouse cells, cultured rat cells, Chinese hamster ovary (CHO) cells, *Xenopus* oocytes, rabbit reticulocytes, wheat germ, or rye embryo (see, e.g., Krieg & Melton, 1984, Nature 308:203 and Dignam et al., 1990 Methods Enzymol. 182:194-203). Alternatively, the cell-free translation extract, e.g., rabbit reticulocyte lysates and wheat germ extract, can be purchased from, e.g., Promega, (Madison, Wis.). In a preferred embodiment, the cell-free extract is an extract isolated from human cells. In a specific embodiment, the human cells are HeLa cells, lymphocytes, or chondrocytes.

[0424] In addition to the ability to modulate the expression levels of RNA and/or protein products a biomarker of the invention, it may be desirable, at least in certain instances, that compounds modulate the activity of a protein product of a biomarker of the invention. Thus, the present invention provides methods of identifying compounds to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, comprising methods for identifying compounds that modulate the activity of a protein product of one or more biomarkers of the invention. Such methods can comprise: (a) contacting a cell expressing a protein product of one or more biomarkers of the invention with a test compound; (b) determining the activity level of the protein product; and (c) comparing the activity level to that in a corresponding control cell that has not been contacted with the test compound, so that if the level of activity in (a) is altered relative to the level of activity in the control cell, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. In a specific embodiment, the activity level(s) is altered by 5%, 10%, 15%, 25%, 30%, 40%, 50%, 5 to 25%, 10 to 30%, at least 1 fold, at least 1.5 fold, at least 2 fold, 4 fold, 5 fold, 10 fold, 25 fold, 1 to 10 fold, or 5 to 25 fold relative to the activity level in the control as determined by utilizing an assay described herein (e.g., a microarray or RT-PCR) or an assay well known to one of skill in the art. In alternate embodiments, such a method comprises deteiuiining the activity level of a protein product of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, 1 to 5, 1-10, 5-10, 5-25, or 10-40, all or any combination of the biomarkers of the invention present in the cell and comparing the activity levels to those present in the control.

[0425] The present invention provides methods of identifying compounds to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, comprising: (a) contacting a cell-free extract with a nucleic acid encoding a protein product of one or more biomarkers of the invention and a test compound; (b) determining the activity level of the protein product; and (c) comparing the activity level to that in a corresponding control that has not been contacted with the test compound, so that if the level of activity in (a) is altered relative to the level of activity in the control, a compound to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is identified. In a specific embodiment, the activity level(s) is altered by 5%, 10%, 15%, 25%, 30%, 40%, 50%, 5 to 25%, 10 to 30%, at least 1 fold, at least 1.5 fold, at least 2 fold, 4 fold, 5 fold, 10 fold, 25 fold, 1 to 10 fold, or 5 to 25 fold relative to the activity level in the control as determined by utilizing an assay described herein (e.g., a microarray or RT-PCR) or an assay well known to one of skill in the art. In alternate embodiments, such a method comprises determining the activity level of a protein product of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, 1 to 5, 1-10, 5-10, 5-25, or 10-40, all or any combination of the biomarkers of the invention present in the sample and comparing the activity levels to those present in the control.

[0426] Standard techniques can be utilized to determine the level of activity of a protein product of a biomarker of the invention. See, e.g., Table 5, infra, for examples of activities

of protein products of biomarkers of the invention that can be determined using techniques well known in the art.

TABLE 5

Gene Symbol	Activity
ABCA1	Regulation of cholesterol and phospholipid efflux
ABCG1	Regulation of cholesterol and phospholipid efflux
BCL6	Regulation of T cell and B cell interactions; the Expression of B7-1/CD80
ACP1	Regulation of cell motility; Interaction with and dephosphorylation of FAK
ADPRT	Interaction with proliferating cell nuclear antigen (PCNA); Transfer of ADP-ribose moieties to itself and to nuclear acceptor proteins
ANGPTL2	Sprouting in vascular endothelial cells
B2M	Interaction with MHC1 alpha 3 domain
BMPR2	Phosphorylation of Smads 1, 5, and 8
CLIC4	Interaction with ERK7
Egr1	LDLR transcription; Activation of human PPARgammal gene expression
IKBKAP	Interaction with NF-kappaB-inducing kinase (NIK) and IKB kinases (IKKs)
IL13RA1	Phosphorylation of IL13RA1; Binding to Tyk2; Activation of Jak1, Tyk2, the insulin receptor substrate-1 and STAT6
ILF1	Binding to the interleukin-2 (IL-2) promoter; Regulation of IL-2 gene expression
NCOA1	Association with STAT3
PAIP2	Interaction with PABP
PDCD5	Translocation to the nucleus
PDK4	Phosphorylation of pyruvate dehydrogenase complex (PDC)
PF4	Binding to Heparin; Anti-heparin activities
SETBP1	Interaction with SET
TSG-6	Binding to hyaluronan and glycosaminoglycans
TNFAIP6	Role in extracellular remodeling and cellular proliferation
Yes1	Tyrosine kinase activity

5.20.2 Biological Activity of the Compounds

[0427] Upon identification of compounds to be tested for an ability to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof (for convenience referred to herein as a "lead" compound), the compounds can be further investigated. For example, the compounds identified via the present methods can be further tested in vivo in accepted animal models of inflammation, preferably, arthritis and more preferably, osteoarthritis. Further, the compounds identified via the methods can be analyzed with respect to their specificity. In particular, the compounds can be tested for an effect on manufacture of type II collagen and proteoglycans by chondrocytes. Techniques for such additional compound investigation are well known to one of skill in the art.

[0428] In one embodiment, the effect of a lead compound can be assayed by measuring the cell growth or viability of the target cell. Such assays can be carried out with representative cells of cell types involved in osteoarthritis (e.g., chondrocytes). Alternatively, instead of culturing cells from a patient, a lead compound may be screened using cells of a cell line.

[0429] Many assays well-known in the art can be used to assess the survival and/or growth of a patient cell or cell line following exposure to a lead compound; for example, cell proliferation can be assayed by measuring Bromodeoxyuridine (BrdU) incorporation (see, e.g., Hoshino et al., 1986, Int. J. Cancer 38, 369; Campana et al., 1988, J. Immunol. Meth. 107:79) or (^{3}H)-thymidine incorporation (see, e.g., Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73), by direct cell count, by detecting changes in transcription, translation or activity of known genes such as proto-oncogenes (e.g., fos, myc) or cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc). The levels of such protein and RNA (e.g., mRNA) and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as Western blotting or immunoprecipitation using commercially available antibodies. mRNA can be quantitated using methods that are well known and routine in the art, for example, using northern analysis, RNase protection, the polymerase chain reaction in connection with the reverse transcription. Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art. In a specific embodiment, the level of cellular ATP is measured to determined cell viability. Differentiation can be assessed, for example, visually based on changes in morphology.

[0430] One example of a chondrocyte proliferation assay is as follows: Chondrocytes are retrieved from human severe OA cartilage slices as previously described. (Doherty P J, Zhang H, Trembley L, Manolopoulos V and Marshall K W., 1998, Osteoarthritis and Cartilage 6:153-160). Cells are then washed, counted and seeded at 1×10^4 cells/well in a flat-bottomed 96-well plate (Corning) in DMEM++. After cells attach to the plate, they are washed with DMEM only, and then incubated in DMEM with or without 10% FCS along with different concentrations of lead compound for 48 hours. The cell number in each well is then determined by adding 10 μl of WST-1 (a tetrazolium salt that can be cleaved to formazan by mitochondrial dehydrogenases in live cells, Roche) to each well, mixing thoroughly for 1 min. and incubating at 37° for 1.5 hours. Then the plate is scanned by a microplate autoreader (BIO-TEK Instruments) at an absorbance of 450 nm. The number of viable cells is reflected by the amount of formazan formed which is quantified by measuring absorbance at 450 nm. (Lang I, Hoffmann C, Olip H, Pabst M A, Hahn T, Dohr G, Desoye G., 2001, Differential mitogenic responses of human macrovascular and microvascular endothelial cells to cytokines underline their phenotypic heterogeneity. Cell Prolif 34:143-55).

[0431] The effect on manufacture of type II collagen and proteoglycans by chondrocytes exposed to a lead compound can be determined using techniques well known in the art. Further, any assay well known in the art for assessing the efficacy of a therapy for prevention, treatment, management or amelioration of a condition, in particular osteoarthritis, can be performed using the lead compounds.

Animal Models

[0432] Compounds can be tested in suitable animal model systems prior to use in humans. Such animal model systems include but are not limited to rats, mice, chicken, cows, monkeys, pigs, dogs, rabbits, etc. Any animal system well-known in the art may be used. In certain embodiments, compounds are tested in a mouse model. Compounds can be administered repeatedly.

[0433] Accepted animal models can be utilized to determine the efficacy of the compounds identified via the methods

described above for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof. Such models can include the various experimental animal models of inflammatory arthritis known in the art and described in Crofford L. J. and Wilder R. L., "Arthritis and Autoimmunity in Animals", in Arthritis and Allied Conditions: A Textbook of Rheumatology, McCarty et al. (eds.), Chapter 30 (Lee and Febiger, 1993). The principle animal models for arthritis or inflammatory disease known in the art and widely used include: adjuvant-induced arthritis rat models, collagen-induced arthritis rat and mouse models and antigen-induced arthritis rat, rabbit and hamster models, all described in Crofford L. J. and Wilder R. L., "Arthritis and Autoimmunity in Animals", in Arthritis and Allied Conditions: A Textbook of Rheumatology, McCarty et al. (eds.), Chapter 30 (Lee and Febiger, 1993), incorporated herein by reference in its entirety.

[0434] In one embodiment, the efficacy of a compound for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof is determined using a carrageenan-induced arthritis rat model. Carrageenan-induced arthritis has also been used in rabbit, dog and pig in studies of chronic arthritis or inflammation. Quantitative histomorphometric assessment is used to determine therapeutic efficacy. The methods for using such a carrageenan-induced arthritis model is described in Hansra P. et al., "Carrageenan-Induced Arthritis in the Rat," Inflammation, 24(2): 141-155, (2000). Also commonly used are zymosan-induced inflammation animal models as known and described in the art.

[0435] The anti-inflammatory activity of the compounds can be assessed by measuring the inhibition of carrageenan-induced paw edema in the rat, using a modification of the method described in Winter C. A. et al., "Carrageenan-Induced Edema in Hind Paw of the Rat as an Assay for Anti-inflammatory Drugs" Proc. Soc. Exp. Biol Med. 111, 544-547, (1962). This assay has been used as a primary in vivo screen for the anti-inflammatory activity of most NSAIDs, and is considered predictive of human efficacy. The anti-inflammatory activity of the test compound is expressed as the percent inhibition of the increase in hind paw weight of the test group relative to the vehicle dosed control group.

[0436] In another embodiment, the efficacy of a compound for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof is determined using a collagen-induced arthritis (CIA) model. CIA is an animal model for the human autoinunune disease rheumatoid arthritis (RA) (Trenthorn et al., 1977, J. Exp. Med., 146 :857). This disease can be induced in many species by the administration of heterologous type II collagen (Courtenay et al., 1980, Nature 283:665; Cathcart et at, 1986, Lab. Invest., 54:26). With respect to animal models of arthritis see, in addition, e.g., Holmdahl, R., 1999, Curr. Biol. 15:R528-530.

[0437] In another embodiment, the efficacy of a compound for the prevention, treatment, management and/or amelioration of osteoarthritis or a symptom thereof is determined using assays that determine bone formation and/or bone loss. Animal models such as ovariectomy-induced bone resorption mice, rat and rabbit models are known in the art for obtaining dynamic parameters for bone formation. Using methods such as those described by Yositake et al. or Yamamoto et al, bone volume is measured in vivo by microcomputed tomography analysis and bone histomorphometry analysis. Yoshitake et al., "Osteopontin-Deficient Mice Are Resist ant to Ovariectomy-Induced Bone Resorption," Proc. Natl. Acad. Sci. 96:8156-8160, (1999); Yamamoto et al., "The Integrin Ligand Echistatin Prevents Bone Loss in Ovariectomized Mice and Rats," Endocrinology 139(3):1411-1419, (1998), both incorporated herein by reference in their entirety.

Toxicity

[0438] The toxicity and/or efficacy of a compound identified in accordance with the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). Cells and cell lines that can be used to assess the cytotoxicity of a compound identified in accordance with the invention include, but are not limited to, peripheral blood mononuclear cells (PBMCs), Caco-2 cells, and Huh7 cells. The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. A compound identified in accordance with the invention that exhibits large therapeutic indices is preferred. While a compound identified in accordance with the invention that exhibits toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0439] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of a compound identified in accordance with the invention for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Design of Congeners or Analogs

[0440] The compounds which display the desired biological activity can be used as lead compounds for the development or design of congeners or analogs having useful pharmacological activity. For example, once a lead compound is identified, molecular modeling techniques can be used to design variants of the compound that can be more effective. Examples of molecular modeling systems are the CHARM and QUANTA programs (Polygen Corporation, Waltham, Mass.). CHARM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

[0441] A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen et al., 1988, Acta Pharmaceutical Fennica 97:159-166; Ripka, 1998, New Scientist 54-57; McKinaly & Rossmann, 1989, Annu. Rev. Pharmacol. Toxiciol. 29:111-122; Perry &

Davies, OSAR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis & Dean, 1989, Proc. R. Soc. Lond. 236:125-140 and 141-162; Askew et al., 1989, J. Am. Chem. Soc. 111:1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, Calif.), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to any identified region. The analogs and congeners can be tested for binding to the proteins of interest (i.e., the protein products of a biomarker of the invention) using the above-described screens for biologic activity. Alternatively, lead compounds with little or no biologic activity, as ascertained in the screen, can also be used to design analogs and congeners of the compound that have biologic activity.

5.20.3 Compounds

[0442] Compounds that can be tested and identified methods described herein can include, but are not limited to, compounds obtained from any commercial source, including Aldrich (1001 West St. Paul Ave., Milwaukee, Wis. 53233), Sigma Chemical (P.O. Box 14508, St. Louis, Mo. 63178), Fluka Chemie AG (Industriestrasse 25, CH-9471 Buchs, Switzerland (Fluka Chemical Corp. 980 South 2nd Street, Ronkonkoma, N.Y. 11779)), Eastman Chemical Company, Fine Chemicals (P.O Box 431, Kingsport, Tenn. 37662), Boehringer Mannheim GmbH (Sandhofer Strasse 116, D-68298 Mannheim), Takasago (4 Volvo Drive, Rockleigh, N.J. 07647), SST Corporation (635 Brighton Road, Clifton, N.J. 07012), Ferro (111 West Irene Road, Zachary, La. 70791), Riedel-deHaen Aktiengesellschaft (P.O. Box D-30918, Seelze, Germany), PPG Industries Inc., Fine Chemicals (One PPG Place, 34th Floor, Pittsburgh, Pa. 15272). Further any kind of natural products may be screened using the methods of the invention, including microbial, fungal, plant or animal extracts.

[0443] Compounds from large libraries of synthetic or natural compounds can be screened. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, N.J.), Brandon Associates (Merrimack, N.H.), and Microsource (New Milford, Conn.). A rare chemical library is available from Aldrich (Milwaukee, Wis.). Combinatorial libraries are available and are prepared. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g., Pan Laboratories (Bothell, Wash.) or MycoSearch (NC), or are readily produceable by methods well known in the art. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means.

[0444] Furthermore, diversity libraries of test compounds, including small molecule test compounds, may be utilized. Libraries screened using the methods of the present invention can comprise a variety of types of compounds. Examples of libraries that can be screened in accordance with the methods of the invention include, but are not limited to, peptoids; random biooligomers; diversomers such as hydantoins, benzodiazepines and dipeptides; vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates; peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; carbohydrate libraries; and small molecule libraries (preferably, small organic molecule libraries). In some embodiments, the compounds in the libraries screened are nucleic acid or peptide molecules. In a non-limiting example, peptide molecules can exist in a phage display library. In other embodiments, the types of compounds include, but are not limited to, peptide analogs including peptides comprising non-naturally occurring amino acids, e.g., D-amino acids, phosphorous analogs of amino acids, such as α-amino phosphoric acids and α-amino phosphoric acids, or amino acids having non-peptide linkages, nucleic acid analogs such as phosphorothioates and PNAs, hormones, antigens, synthetic or naturally occurring drugs, opiates, dopamine, serotonin, catecholamines, thrombin, acetylcholine, prostaglandins, organic molecules, pheromones, adenosine, sucrose, glucose, lactose and galactose. Libraries of polypeptides or proteins can also be used in the assays of the invention.

[0445] In a specific embodiment, the combinatorial libraries are small organic molecule libraries including, but not limited to, benzodiazepines, isoprenoids, thiazolidinones, metathiazanones, pyrrolidines, morpholino compounds, and benzodiazepines. In another embodiment, the combinatorial libraries comprise peptoids; random bio-oligomers; benzodiazepines; diversomers such as hydantoins, benzodiazepines and dipeptides;, vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates; peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; or carbohydrate libraries. Combinatorial libraries are themselves commercially available For example, libraries may be commercially obtained from, e.g., Specs and BioSpecs B.V. (Rijswijk, The Netherlands), Chembridge Corporation (San Diego, Calif.), Contract Service Company (Dolgoprudny, Moscow Region, Russia), Comgenex USA Inc. (Princeton, N.J.), Maybridge Chemicals Ltd. (Cornwall PL34 OHW, United Kingdom), Asinex (Moscow, Russia), ComGenex (Princeton, N.J.), Ru, Tripos, Inc. (St. Louis, Mo.), ChemStar, Ltd (Moscow, Russia), 3D Pharmaceuticals (Exton, Pa.), and Martek Biosciences (Columbia, Md.).

[0446] In a preferred embodiment, the library is preselected so that the compounds of the library are more amenable for cellular uptake. For example, compounds are selected based on specific parameters such as, but not limited to, size, lipophilicity, hydrophilicity, and hydrogen bonding, which enhance the likelihood of compounds getting into the cells. In another embodiment, the compounds are analyzed by three-dimensional or four-dimensional computer computation programs.

[0447] The combinatorial compound library for use in accordance with the methods of the present invention may be synthesized. There is a great interest in synthetic methods directed toward the creation of large collections of small organic compounds, or libraries, which could be screened for pharmacological, biological or other activity. The synthetic methods applied to create vast combinatorial libraries are performed in solution or in the solid phase, i.e., on a solid support. Solid-phase synthesis makes it easier to conduct multi-step reactions and to drive reactions to completion with high yields because excess reagents can be easily added and washed away after each reaction step. Solid-phase combinatorial synthesis also tends to improve isolation, purification

and screening. However, the more traditional solution phase chemistry supports a wider variety of organic reactions than solid-phase chemistry.

[0448] Combinatorial compound libraries of the present invention may be synthesized using the apparatus described in U.S. Pat. No. 6,190,619 to Kilcoin et al., which is hereby incorporated by reference in its entirety. U.S. Pat. No. 6,190,619 discloses a synthesis apparatus capable of holding a plurality of reaction vessels for parallel synthesis of multiple discrete compounds or for combinatorial libraries of compounds.

[0449] In one embodiment, the combinatorial compound library can be synthesized in solution. The method disclosed in U.S. Pat. No. 6,194,612 to Boger et al., which is hereby incorporated by reference in its entirety, features compounds useful as templates for solution phase synthesis of combinatorial libraries. The template is designed to permit reaction products to be easily purified from unreacted reactants using liquid/liquid or solid/liquid extractions. The compounds produced by combinatorial synthesis using the template will preferably be small organic molecules. Some compounds in the library may mimic the effects of non-peptides or peptides. In contrast to solid phase synthesize of combinatorial compound libraries, liquid phase synthesis does not require the use of specialized protocols for monitoring the individual steps of a multistep solid phase synthesis (Egner et al., 1995, J. Org. Chem. 60:2652; Anderson et al., 1995, J. Org. Chem. 60:2650; Fitch et al., 1994, J. Org. Chem. 59:7955; Look et al., 1994, J. Org. Chem. 49:7588; Metzger et al., 1993, Angew. Chem., Int. Ed. Engl. 32:894; Youngquist et al., 1994, Rapid Conunun. Mass Spect. 8:77; Chu et al., 1995, J. Am. Chem. Soc. 117:5419; Brummel et al., 1994, Science 264: 399; and Stevanovic et al., 1993, Bioorg. Med. Chem. Len. 3:431).

[0450] Combinatorial compound libraries useful for the methods of the present invention can be synthesized on solid supports. In one embodiment, a split synthesis method, a protocol of separating and mixing solid supports during the synthesis, is used to synthesize a library of compounds on solid supports (see e.g., Lam et al., 1997, Chem. Rev. 97:41-448; Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926 and references cited therein). Each solid support in the final library has substantially one type of compound attached to its surface. Other methods for synthesizing combinatorial libraries on solid supports, wherein one product is attached to each support, will be known to those of skill in the art (see, e.g., Nefzi et al., 1997, Chem. Rev. 97:449-472).

[0451] In some embodiments of the present invention, compounds can be attached to solid supports via linkers. Linkers can be integral and part of the solid support, or they may be nonintegral that are either synthesized on the solid support or attached thereto after synthesis. Linkers are useful not only for providing points of compound attachment to the solid support, but also for allowing different groups of molecules to be cleaved from the solid support under different conditions, depending on the nature of the linker. For example, linkers can be, inter alia, electrophilically cleaved, nucleophilically cleaved, photocleavable, enzymatically cleaved, cleaved by metals, cleaved under reductive conditions or cleaved under oxidative conditions. In a preferred embodiment, the compounds are cleaved from the solid support prior to high throughput screening of the compounds.

[0452] If the library comprises arrays or microarrays of compounds, wherein each compound has an address or identifier, the compound can be deconvoluted, e.g., by cross-referencing the positive sample to original compound list that was applied to the individual test assays.

[0453] If the library is a peptide or nucleic acid library, the sequence of the compound can be determined by direct sequencing of the peptide or nucleic acid. Such methods are well known to one of skill in the art.

[0454] A number of physico-chemical techniques can be used for the de novo characterization of compounds. Examples of such techniques include, but are not limited to, mass spectrometry, NMR spectroscopy, X-ray crytallography and vibrational spectroscopy.

5.21 Use of Identified Compounds to Prevent, Treat, Manage or Ameliorate Osteoarthritis or a Symptom Thereof

[0455] The present invention provides methods of preventing, treating, managing or ameliorating osteoarthritis or a symptom thereof, said methods comprising administering to a subject in need thereof one or more compounds identified in accordance with the methods of the invention. In certain embodiments, the subject has mild, moderate, marked or severe osteoarthritis. In a preferred embodiment, the subject is human.

[0456] In one embodiment, the invention provides a method of preventing, treating, managing or ameliorating osteoarthritis or a symptom thereof, said method comprising administering to a subject in need thereof a dose of a prophylactically or therapeutically effective amount of one or more compounds identified in accordance with the methods of the invention. In a specific embodiment, a compound identified in accordance with the methods of the invention is not administered to prevent, treat, or ameliorate osteoarthritis or a symptom thereof, if such compound has been used previously to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In another embodiment, a compound identified in accordance with the methods of the invention is not administered to prevent, treat, or ameliorate osteoarthritis or a symptom thereof, if such compound has suggested to be used to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In another embodiment, a compound identified in accordance with the methods of the invention specifically binds to and/or alters the expression and/or activity level of a protein or RNA product of only one biomarker of the invention. In another embodiment, a compound identified in accordance with the methods of the invention is not administered to prevent, treat, or ameliorate osteoarthritis or a symptom thereof, if such compound binds to and/or alters the expression and/or activity of a protein or RNA product of one, two, three, all or any combination of the following biomarkers: B2M, TNFAIP6, PDCD5, and EGR1. In yet another embodiment, a compound identified in accordance with the methods of the invention binds to and/or alters the expression and/or activity level of a protein or RNA product of at least 2, at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40 or more biomarkers of the invention.

[0457] In a specific embodiment, a compound identified in accordance with the methods of the invention increases or decreases the anabolic and/or the catabolic activity of a chondrocyte. Preferably, such a compound increases or decreases the anabolic and/or catabolic activity of a chondrocyte by

greater than 1.0-fold, more preferably, 1.5-5-fold, and most preferably, 5-100-fold, as compared to an untreated chondrocyte. In another embodiment, a compound identified in accordance with the methods of the invention ameliorates at least one of the symptoms and/or changes associated with osteoarthritis including cartilage degeneration, or pain, swelling, weakness and/or loss of functional ability in the afflicted joints, associated with cartilage degeneration. In a particular embodiment, the prophylactic or therapeutic agent administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof is a synthetic compound or a natural product (e.g. a plant extract or culture supernatant), or a mixture of compounds.

[0458] The invention also provides methods of preventing, treating, managing or ameliorating osteoarthritis or a symptom thereof, said methods comprising administering to a subject in need thereof one or more of the compounds identified utilizing the screening methods described herein, and one or more other therapies (e.g., prophylactic or therapeutic agents and surgery). In a specific embodiment, such therapies are currently being used, have been used or are known to be useful in the prevention, treatment, management or amelioration of osteoarthritis or a symptom thereof (including, but not limited to the prophylactic or therapeutic agents listed in Section 5.21.2 hereinbelow). The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the invention can be administered sequentially or concurrently. In a specific embodiment, the combination therapies of the invention comprise a compound identified in accordance with the invention and at least one other therapy that has the same mechanism of action as said compound. In another specific embodiment, the combination therapies of the invention comprise a compound identified in accordance with the methods of the invention and at least one other therapy (e.g., prophylactic or therapeutic agent) which has a different mechanism of action than said compound. The combination therapies of the present invention improve the prophylactic or therapeutic effect of a compound of the invention by functioning together with the compound to have an additive or synergistic effect. The combination therapies of the present invention reduce the side effects associated with the therapies (e.g., prophylactic or therapeutic agents).

[0459] The prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

[0460] In specific embodiment, a pharmaceutical composition comprising one or more compounds identified in an assay described herein is administered to a subject, preferably a human, to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In accordance with the invention, the pharmaceutical composition may also comprise one or more prophylactic or therapeutic agents. Preferably, such agents are currently being used, have been used or are known to be useful in the prevention, treatment, management or amelioration of osteoarthritis or a symptom thereof.

[0461] A compound identified in accordance with the methods of the invention may be used as a first, second, third, fourth or fifth line of therapy for osteoarthritis. The invention provides methods for treating, managing or ameliorating osteoarthritis or a symptom thereof in a subject refractory to conventional therapies for osteoarthritis, said methods comprising administering to said subject a dose of a prophylactically or therapeutically effective amount of a compound identified in accordance with the methods of the invention.

[0462] The invention provides methods for treating, managing or ameliorating osteoarthritis or a symptom thereof in a subject refractory to existing single agent therapies for osteoarthritis, said methods comprising administering to said subject a dose of a prophylactically or therapeutically effective amount of a compound identified in accordance with the methods of the invention and a dose of a prophylactically or therapeutically effective amount of one or more other therapies (e.g., prophylactic or therapeutic agents). The invention also provides methods for treating or managing a osteoarthritis by administering a compound identified in accordance with the methods of the invention in combination with any other therapy (e.g., surgery) to patients who have proven refractory to other therapies but are no longer on these therapies. The invention also provides methods for the treatment or management of a patient having osteoarthritis and immunosuppressed by reason of having previously undergone other therapies. The invention also provides alternative methods for the treatment or management of osteoarthritis where hormonal therapy and/or biological therapy/immunotherapy has proven or may prove too toxic, i.e., results in unacceptable or unbearable side effects, for the subject being treated or managed.

5.21.1 Compounds for Use in Preventing, Treating, Managing or Ameliorating Osteoarthritis or a Symptom Thereof

[0463] Representative, non-limiting examples of compounds that can used in accordance with the methods of the invention to prevent, treat, manage and/or ameliorate osteoarthritis or a symptom thereof are described in detail below.

[0464] First, such compounds can include, for example, antisense, ribozyme, or triple helix compounds that can downregulate the expression or activity of a protein or RNA product of a biomarker of the invention. Such compounds are described in detail in the subsection below.

[0465] Second, such compounds can include, for example, antibody compositions that can modulate the expression of a protein or RNA product of a biomarker of the invention, or the activity of a protein product of a biomarker of the invention. In a specific embodiment, the antibody compositions downregulate the expression a protein or RNA product of a biomarker of the invention, or the activity of a protein product of a biomarker of the invention. Such compounds are described in detail in the subsection below.

[0466] Third, such compounds can include, for example, protein products of a biomarker of the invention. The invention encompasses the use of peptides or peptide mimetics selected to mimic a protein product of a biomarker of the invention to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. Further, such compounds can include, for example, dominant-negative polypeptides that can modulate the expression a protein or RNA product of a biomarker of the invention, or the activity of a protein product of a biomarker of the invention.

[0467] The methods also encompasses the use derivatives, analogs and fragments of a protein product of a biomarker of the invention to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In particular, the invention

encompasses the use of fragments of a protein product of a biomarker of the invention comprising one or more domains of such a protein(s) to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In another specific embodiment, the invention encompasses the use of a protein product of a biomarker of the invention, or an analog, derivative or fragment of such a protein which is expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence).

[0468] In specific embodiments, an antisense oligonucleotide of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, or more of biomarkers of the invention are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In other embodiments, one or more of protein products of a biomarker of the invention or a fragment, analog, or derivative thereof are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In other embodiment, one or more antibodies that specifically bind to a protein product of the invention are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In other embodiments, one or more dominant-negative polypeptides are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof.

Antisense, Ribozyme, Triple-Helix Compositions

[0469] Representative, non-limiting examples of antisense molecules include those listed in Table 6.

TABLE 6

Gene Symbol	Examples of Antisense Sequences	SEQ ID NO:
B2M	5'-TTAAAGGACTTAACGATACA-3'	1
BCL6	5'-TGTAGAGCCGAGTTAAACGC-3'	2
C1QR1	5'-GCTCTGAGTCTCAGTAATAA-3'	3
CCNC	5'-TAAGATACGGTCCATAAGAG-3'	4
EBNA1BP2	5'-CTTCGTTACAAACCGTCGGA-3'	5
FLJ32234	5'-CTTTTACACTTCTATGGAAG-3	6
G2AN	5'-TCAGAGTGACCCTGGGTCCG-3'	7
HSPCA	5'-CGGAAAGTCCGTCTTTAACG-3'	8
IKBKAP	5'-ACCTAGTCCTCAGACACACA-3'	9
IL13RA1	5'-CTCACTCTTCGGATCGTAAA-3'	10
LAMC1	5'-TCGTCCGGAACACATGACTA-3'	11
MAFB	5'-GTCCCGTCCCGTCCCTTCGA-3'	12
PAIP2	5'-GTTCGTAGTAGTTACTTCTA-3'	13
PER1	5'-GGTCCTTATGATGGTCGTCA-3'	14
PF4	5'-CAACGACGAGGACGGTGAAC-3'	15
TNFAIP6	5'-TTTTTAACCTAAAGTACAGA-3'	16
WDR9	5'-ACTATCCTGTCCTGTATCTT-3'	17

TABLE 6-continued

Gene Symbol	Examples of Antisense Sequences	SEQ ID NO:
IRF1	5'-TCTAGGGTACCTTCGTACGA-3'	18
ABCA1	5'-CTTCGGTTAGGACTCTTGTG-3'	19
ABCG1	5'-TCTGACGCACAGGACGTTTT-3'	20
NCOA1	5'-GGTCGATAATGCCCACATCT-3'	21
CLIC4	5'-GTGCATTTAAAGACCTACCG-3'	22
SFRS6	5'-CCACCTATGTCGTCAGCCTC-3'	23
HSPCB	5'-CTTCTGGTGAACCGTCAGTT-3'	24
ADPRT	5'-CACTTCCGGTACTAACTCTT-3'	25
ACP1	5'-ACAGCTAGTGGGTAACGTCT-3'	26
PMSCL2	5'-GAAGGGCCTGCGGCTGTCGA-3'	27
FLJ13612	5'-GTCGGCGGCGGAGTAGTTCC-3'	28
SLC5A6	5'-TCTCTAGATGGCTAAACCCT-3'	29
YES1	5'-ACCTTGGTGCTTTCATCGTT-3'	30
CLN3	5'-AGAGATGCCGACGACACGAG-3'	31
LCMT2	5'-GTTGTAAAAGCCGTCGATTT-3'	32
NXN	5'-AGTCCCTTCAGTAACGTCCC-3'	33
ANGPTL2	5'-TCTGCATGTTCGTTCCCAAA-3'	34
BMPR2	5'-ATCAACCTCTACTCTCTCAG-3'	35
CLECSF6	5'-ATTATGGCCTAAGGGGTTCG-3'	36
DNAPTP6	5'-GTGTCTTCCGTTGTCTGATG-3'	37
F2RL1	5'-GAACTTCTAACGGATAGTGT-3'	38
FLJ11000	5'-GTTGTCACTACTCTCTCGCA-3'	39
FLJ11142	5'-TTAACCGCAGTAACCCCAAG-3'	40
ILF1	5'-TGCGGGGGCCCGCCGCCGCC-3'	41
PDCD5	5'-CTCTTTGTCATAGAATCGGG-3'	42
PDK4	5'-AGTTGCCGCGGCCGGACCAC-3'	43
PPIF	5'-CCCGAGGCCGCTGGGCAGGA-3'	44
SETBP1	5'-TGGAGTTTCCTAAAGTCGGT-3'	45
ZFR	5'-GTCGTTGACTGATACCAATA-3'	46
EGR1	5'-TGCGGCTTGTGACTGTAAAA-3'	47
TSPAN2	5'-CTCATAAAGATACACCCCGA-3'	48

[0470] In addition, standard techniques can be utilized to produce antisense, triple helix, or ribozyme molecules for use as part of the methods described herein. First, standard techniques can be utilized for the production of antisense nucleic acid molecules, i.e., molecules which are complementary to a sense nucleic acid encoding a polypeptide of interest, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence.

Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding a polypeptide of interest. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences that flank the coding region and are not translated into amino acids.

[0471] An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides or more in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl)uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

[0472] Antisense nucleic acid molecules administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA encoding the polypeptide of interest to thereby inhibit expression, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue, e.g., a joint (e.g., a knee, hip, elbow, and knuckle), site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell, e.g., a T cell or chondrocyte, surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using vectors, e.g., gene therapy vectors, described below. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[0473] An antisense nucleic acid molecule of interest can be an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual α-units, the strands run parallel to each other (Gaultier et al., 1987, Nucleic Acids Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., 1987, Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

[0474] Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region, and can also be generated using standard techniques. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach, 1988, Nature 334:585-591)) can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. A ribozyme having specificity for a nucleic acid molecule encoding a polypeptide of interest can be designed based upon the nucleotide sequence of a cDNA disclosed herein. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a Cech et al. U.S. Pat. No. 4,987,071; and Cech et al. U.S. Pat. No. 5,116,742. Alternatively, an mRNA encoding a polypeptide of interest can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel and Szostak, 1993, Science 261:1411-1418.

[0475] Triple helical structures can also be generated using well known techniques. For example, expression of a polypeptide of interest can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the gene encoding the polypeptide (e.g., the promoter and/or enhancer) to form triple helical structures that prevent transcription of the gene in target cells. See generally Helene, 1991, Anticancer Drug Des. 6(6):569-84; Helene, 1992, Ann. N.Y. Acad. Sci. 660:27-36; and Maher, 1992, Bioassays 14(12):807-15.

[0476] In various embodiments, nucleic acid compositions can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup et al., 1996, Bioorganic & Medicinal Chemistry 4(1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as

described in Hyrup et al., 1996, supra; Perry-O'Keefe et al., 1996, Proc. Natl. Acad. Sci. USA 93: 14670-675.

[0477] PNAs can, for example, be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNAse H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup, 1996, supra). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, 1996, supra, and Finn et al., 1996, Nucleic Acids Res. 24(17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds such as 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite can be used as a link between the PNA and the 5' end of DNA (Mag et al., 1989, Nucleic Acids Res. 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn et al., 1996, Nucleic Acids Res. 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser et al., 1975, Bioorganic Med. Chem. Lett. 5:1119-11124).

[0478] In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in viva), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et a]., 1989, Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. USA 84:648-652; International Publication No. W0 88/09810) or the blood-brain barrier (see, e.g., International Publication No. W0 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol et al., 1988, Bio/Techniques 6:958-976) or intercalating agents (see, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Antibody Compositions

[0479] In one embodiment, antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention are administered to a subject, preferably a human, to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In another embodiment, any combination of antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention are administered to a subject, preferably a human, to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In a specific embodiment, one or more antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention are administered to a subject, preferably a human, in combination with other types of therapies (e.g., NSAIDS) to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In certain embodiments, antibodies known in the art that specifically bind to one or more protein products of one or more biomarkers of the invention are administered to a subject, preferably a human, alone or in combination with other types of therapies (e.g., NSAIDS) to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof. In other embodiments, antibodies known in the art that specifically bind to one or more protein products of one or more biomarkers of the invention are not administered to a subject, preferably a human, alone or in combination with other types of therapies (e.g., NSAIDS) to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof.

[0480] One or more antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention can be administered to a subject, preferably a human, using various delivery systems are known to those of skill in the art. For example, such antibodies can be administered by encapsulation in liposomes, microparticles or microcapsules. See, e.g., U.S. Pat. No. 5,762,904, U.S. Pat. No. 6,004,534, and International Publication No. WO 99/52563. In addition, such antibodies can be administered using recombinant cells capable of expressing the antibodies, or retroviral, other viral vectors or non-viral vectors capable of expressing the antibodies.

[0481] Antibodies that specifically bind one or more protein products of one or more biomarkers of the invention can be obtained from any known source. Alternatively, antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0482] Antibodies include, but are not limited to, polyclonal antibodies, monoclonal antibodies, bispecific antibodies, multispecific antibodies, human antibodies, humanized antibodies, camelised antibodies, chimeric antibodies, single-chain Fvs (scFv) (see e.g., Bird et al. (1988) *Science* 242:423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883), single chain antibodies, single domain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody", as used herein, refers to immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site. Immunoglobulin molecules can he of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass. Examples of immunologically active fragments of immunoglobulin molecules include F(ab) fragments (a monovalent fragment consisting of the VL, VH, CL and CH1 domains) and F(ab')2 fragments (a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region) which can be generated by treating the antibody with an enzyme such as pepsin or papain. Immunologically active fragments also include, but are not limited to, Fd fragments (consisting of the VH and CH1 domains), Fv fragments (consisting of the VL and VH domains of a single arm of an antibody), dAb fragments (consisting of a VH domain; Ward et al., (1989) *Nature* 341: 544-546), and isolated complementarity determining regions (CDRs). Antibodies that specifically bind to an antigen can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0483] Polyclonal antibodies that specifically bind to an antigen can be produced by various procedures well-known in the art. For example, a human antigen can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the human antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0484] The term "monospecific antibody" refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes monoclonal antibodies. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. See, e.g., U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, 4,411,993 and 4,196,265; Kennett et al (eds.), *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses,* Plenum Press (1980); and Harlow and Lane (eds.), *Antibodies. A Laboratory Manual,* Cold Spring Harbor Laboratory Press (1988), which are incorporated herein by reference. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual,* (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). Other techniques that enable the production of antibodies through recombinant techniques (e.g., techniques described by William D. Huse et al., 1989, Science, 246: 1275-1281; L. Sastry et al., 1989, Proc. Natl. Acad. Sci. USA, 86: 5728-5732; and Michelle Alting-Mees et al., Strategies in Molecular Biology, 3: 1-9 (1990) involving a commercial system available from Stratacyte, La Jolla, Calif.) may also be utilized to construct monoclonal antibodies. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0485] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. Briefly, mice can be immunized with a protein product of a biomarker of the invention, and once an immune response is detected, e.g., antibodies specific for the protein are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. Additionally, a RIMMS (repetitive immunization multiple sites) technique can be used to immunize an animal (Kilptrack et al., 1997, Hybridoma 16:381-9, incorporated by reference in its entirety). The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0486] Accordingly, the present invention provides methods of generating antibodies by culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with a protein product of a biomarker of the invention, with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to the protein or protein fragment.

[0487] Antibody fragments which recognize specific epitopes of a protein product of a biomarker of the invention may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art.

[0488] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (e.g., human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular antigen can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., 1995, J. Immunol. Methods 182:41-50; Ames et al., 1995, J. Immunol. Methods 184:177-186; Kettleborough et al. , 1994, Eur. J. Immunol. 24:952-958; Persic et al., 1997, Gene 187:9-18; Burton et al., 1994, Advances in Immunology 57:191-280; PCT Application No. PCT/GB91/O1 134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Pat. Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0489] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in International Publication No. WO 92/22324; Mullinax et al., 1992, Bio-

Techniques 12(6):864-869; Sawai et al., 1995, AJRI 34:26-34; and Better et al., 1988, Science 240:1041-1043 (said references incorporated by reference in their entireties).

[0490] To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, e.g., the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, e.g., human kappa or lambs constant regions. Preferably, the vectors for expressing the VH or VL domains comprise an EF-1α promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The VH and VL domains may also cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, e.g., IgG, using techniques known to those of skill in the art.

[0491] For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Pat. Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0492] Antibodies can also be produced by a transgenic animal. In particular, human antibodies can be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then be bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., International Publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Pat. Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0493] U.S. Pat. No. 5,849,992, for example, describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted-therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

[0494] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, 1985, Science 229:1202; Oi et 1986, BioTechniques 4:214; Gillies et al., 1989, J. Immunol. Methods 125:191-202; and U.S. Pat. Nos. 5,807,715, 4,816,567, 4,816,397, and 6,331,415, which are incorporated herein by reference in their entirety.

[0495] A humanized antibody is an antibody or its variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immuoglobulin. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab').sub.2, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3 and IgG_4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art. The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the import antibody. Such mutations, how-

ever, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%. Humanized antibody can be produced using variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering 7(6):805-814; and Roguska et al. , 1994, PNAS 91:969-973), chain shuffling (U.S. Pat. No. 5,565,332), and techniques disclosed in, e.g ., U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, WO 9317105, Tan et al., 2002, J. Immunol. 169:1119-25, Caldas et al., 2000, Protein Eng. 13(5):353-60, Morea et al., 2000, Methods 20(3):267-79, Baca et al., 1997, J. Biol. Chem. 272(16):10678-84, Roguska et al., 1996, Protein Eng. 9(10):895-904, Couto et al., 1995, Cancer Res. 55 (23 Supp): 5973s-5977s, Couto et al., 1995, Cancer Res. 55(8):1717-22, Sandhu J S, 1994, Gene 150(2):409-10, and Pedersen et al., 1994, J. Mol. Biol. 235(3):959-73. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, Nature 332:323, which are incorporated herein by reference in their entireties.)

[0496] Single domain antibodies, for example, antibodies lacking the light chains, can be produced by methods well-known in the art. See Riechmann et al., 1999, J. Immuno. 231:25-38; Nuttall et al., 2000, Curr. Pharm. Biotechnol. 1(3):253-263; Muylderman, 2001, J. Biotechnol. 74(4):277302; U.S. Pat. No. 6,005,079; and International Publication Nos. WO 94/04678, WO 94/25591, and WO 01/44301, each of which is incorporated herein by reference in its entirety.

[0497] Further, the antibodies that specifically bind to an antigen can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" an antigen using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1989, FASEB J. 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). Such antibodies can be used, alone or in combination with other therapies, in the prevention, treatment, management or amelioration of osteoarthritis or a symptom thereof.

[0498] The invention encompasses polynucleotides comprising a nucleotide sequence encoding an antibody or fragment thereof that specifically binds to an antigen. The invention also encompasses polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides that encode an antibody of the invention.

[0499] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. The nucleotide sequences encoding known antibodies can be determined using methods well known in the art, i.e., nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, fragments, or variants thereof, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0500] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0501] Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0502] Once a polynucleotide encoding an antibody molecule, heavy or light chain of an antibody, or fragment thereof (preferably, but not necessarily, containing the heavy or light chain variable domain) of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well-known in the art.

[0503] In one preferred embodiment, monoclonal antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin (1980, Proc. Natl. Acad. Sci. USA 77:4216-4220), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp (1982, Mol. Biol. 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, and a cell from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

[0504] In addition to the nucleic acid sequence encoding the diversified immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The

selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr$^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[0505] In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr$^-$ CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G.

[0506] For antibodies that include an Fc domain, the antibody production system preferably synthesizes antibodies in which the Fc region is glycosylated. For example, the Fc domain of IgG molecules is glycosylated at asparagine 297 in the CH2 domain. This asparagine is the site for modification with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fcγ receptors and complement C1q (Burton and Woof, 1992, Adv. Immunol. 51:1-84 ; Jefferis et al., 1998, Immunol. Rev. 163:59-76). In a preferred embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other eukaryotic post-translational modifications.

[0507] Once an antibody molecule has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies or fragments thereof may be fused to heterologous polypeptide sequences known in the art to facilitate purification.

Gene Therapy Techniques

[0508] Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0509] In specific embodiments, one or more antisense oligonucleotides for one or more biomarkers of the invention are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, by way of gene therapy. In other embodiments, one or more nucleic acid molecules comprising nucleotides encoding one or more antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, by way of gene therapy. In other embodiments, one or more nucleic acid molecules comprising nucleotides encoding protein products of one or more biomarkers of the invention or analogs, derivatives or fragments thereof, are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, by way of gene therapy. In yet other embodiments, one or more nucleic acid molecules comprising nucleotides encoding one or more dominant-negative polypeptides of one or more protein products of one or more biomarker of the invention are administered to prevent, treat, manage or ameliorate osteoarthritis or a symptom thereof, by way of gene therapy.

[0510] For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5): 155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

[0511] In one aspect, a composition of the invention comprises nucleic acid sequences encoding one or more antibodies that specifically bind to one or more protein products of one or more biomarkers of the invention, said nucleic acid sequences being part of expression vectors that express one or more antibodies in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibodies, said promoter being inducible or constitutive, and, optionally, tissue-specific.

[0512] In another aspect, a composition of the invention comprises nucleic acid sequences encoding dominant-negative polypeptides of one or protein products of one or more biomarkers of the invention, said nucleic acid sequences being part of expression vectors that express dominant-negative polypeptides in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the dominant-negative polypeptides, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the dominant-negative coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the dominant-negative nucleic acids (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

[0513] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic

acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0514] In a specific embodiment, the nucleic acid sequence is directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., International Publication Nos. WO 92/06180 dated Apr. 16, 1992 (Wu et al.); WO 92/22635 dated Dec. 23, 1992 (Wilson et al.); WO92/20316 dated Nov. 26, 1992 (Findeis et al.); WO 93/14188 dated Jul. 22, 1993 (Clarke et al.), WO 93/20221 dated Oct. 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

[0515] For example, a retroviral vector can be used. These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid sequences encoding the antibodies of interest, or proteins of interest or fragments thereof to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest 93:644-651; Kiem et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

[0516] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, Science 252:431-434; Rosenfeld et al., 1992, Cell 68:143-155; Mastrangeli et al., 1993, J. Clin. Invest. 91:225-234; PCT Publication WO94/12649; and Wang, et al., 1995, Gene Therapy 2:775-783. In a preferred embodiment, adenovirus vectors are used.

[0517] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; U.S. Pat. No. 5,436,146).

[0518] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0519] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0520] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) and/or chondrocytes are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0521] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, chondrocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0522] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0523] In one embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding antibodies of interest, or proteins of interest or fragments thereof are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained

in vitro can potentially be used in accordance with this embodiment of the present invention (see, e.g., International Publication No. WO 94/08598, dated Apr. 28, 1994; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

[0524] Promoters that may be used to control the expression of nucleic acid sequences encoding antibodies of interest, proteins of interest or fragments thereof may be constitutive, inducible or tissue- specific. Non-limiting examples include the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. USA 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff et al., 1978, Proc. Natl. Acad. Sci. USA 75:3727-3731), or the tac promoter (DeBoer et al., 1983, Proc. Natl. Acad. Sci. USA 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

[0525] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

5.21.2 Anti-Inflammatory Therapies

[0526] Anti-inflammatory agents have exhibited success in the treatment, management and amelioration of osteoarthritis and are now a common and a standard therapy for such disorder. Any anti-inflammatory agent well-known to one of skill in the art can be used in the compositions and methods of the invention. Non-limiting examples of anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, beta-agonists, anticholingeric agents, and methyl xanthines. Examples of NSAIDs include, but are not limited to, aspirin, ibuprofen, celecoxib (CELEBREX™), diclofenac (VOLTAREN™), etodolac (LODINE™), fenoprofen (NALFON™), indomethacin (INDOCIN™), ketoralac (TORADOL™), oxaprozin (DAYPRO™), nabumentone (RELAFEN™), sulindac (CLINORIL™), tolmentin (TOLECTIN™), rofecoxib (VIOXX™), naproxen (ALEVE™, NAPROSYN™), ketoprofen (ACTRON™) and nabumetone (RELAFEN™). Such NSAIDs function by inhibiting a cyclooxgenase enzyme (e.g., COX-1 and/or COX-2). Examples of steroidal anti-inflammatory drugs include, but are not limited to, glucocorticoids, dexamethasone (DECADRON™), cortisone, hydrocortisone, prednisone (DELTASONE™), prednisolone, triamcinolone, azulfidine, and eicosanoids such as prostaglandins, thromboxanes, and leukotrienes.

5.22 Pharmaceutical Compositions

[0527] Biologically active compounds identified using the methods of the invention or a pharmaceutically acceptable salt thereof can be administered to a patient, preferably a mammal, more preferably a human, suffering from osteoarthritis. In a specific embodiment, a compound or pharmaceutically acceptable salt thereof is administered to a patient, preferably a mammal, more preferably a human, suffering from the following stage of osteoarthritis: mild, moderate, marked or severe. In another embodiment, a compound or a pharmaceutically acceptable salt thereof is administered to a patient, preferably a mammal, more preferably a human, as a preventative measure against osteoarthritis. In accordance with these embodiments, the patient may be a child, an adult or elderly, wherein a "child" is a subject between the ages of 24 months of age and 18 years of age, an "adult" is a subject 18 years of age or older, and "elderly" is a subject 65 years of age or older.

[0528] When administered to a patient, the compound or a pharmaceutically acceptable salt thereof is preferably administered as component of a composition that optionally comprises a pharmaceutically acceptable vehicle. The composition can be administered orally, or by any other convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal, and intestinal mucosa, etc.) and may be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer the compound and pharmaceutically acceptable salts thereof.

[0529] Methods of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. The mode of administration is left to the discretion of the practitioner. In most instances, administration will result in the release of the compound or a pharmaceutically acceptable salt thereof into the bloodstream.

[0530] In specific embodiments, it may be desirable to administer the compound or a pharmaceutically acceptable salt thereof locally. This may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In a specific embodiment, a compound is administered locally to a joint affected by osteoarthritis.

[0531] In certain embodiments, it may be desirable to introduce the compound or a pharmaceutically acceptable salt thereof into the central nervous system by any suitable route, including intraventricular, intrathecal and epidural injection. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Onunaya reservoir.

[0532] Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the compound and pharmaceutically acceptable salts thereof can be formulated as a suppository, with traditional binders and vehicles such as triglycerides.

[0533] In another embodiment, the compound and pharmaceutically acceptable salts thereof can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

[0534] In yet another embodiment, the compound and pharmaceutically acceptable salts thereof can be delivered in a controlled release system (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, Science 249:1527-1533 may be used. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J. Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled-release system can be placed in proximity of a target RNA of the compound or a pharmaceutically acceptable salt thereof, thus requiring only a fraction of the systemic dose.

[0535] The compounds described herein can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the active compound and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0536] The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a polypeptide or nucleic acid of interest. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of a polypeptide or nucleic acid of interest. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of a polypeptide or nucleic acid of interest and one or more additional active compounds.

[0537] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Intravenous administration is preferred. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0538] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dis-

persion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0539] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0540] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

[0541] Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0542] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0543] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0544] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0545] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegadable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0546] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0547] For antibodies, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (more preferably, 0.1 to 20 mg/kg, 0.1-10 mg/kg, or 0.1 to to 1.0 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al. (1997, J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

[0548] In a specific embodiment, an effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 0.1 to 1.0 mg/kg, 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

[0549] The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

[0550] In addition to those compounds described above, the present invention encompasses the use of small molecules that modulate expression or activity of a nucleic acid or polypeptide of interest. Non-limiting examples of small molecules include peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic

compounds (i.e,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[0551] It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to a subject (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[0552] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

5.23 Kits

[0553] The present invention provides kits for measuring the expression of the protein and RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention. Such kits comprise materials and reagents required for measuring the expression of such protein and RNA products. In specific embodiments, the kits may further comprise one or more additional reagents employed in the various methods, such as: (1) reagents for purifying RNA from blood, chondrocytes or synovial fluid; (2) primers for generating test nucleic acids; (3) dNTPs and/or rNTPs (either premixed or separate), optionally with one or more uniquely labeled dNTPs and/or rNTPs (e.g., biotinylated or Cy3 or Cy5 tagged dNTPs); (4) post synthesis labeling reagents, such as chemically active derivatives of fluorescent dyes; (5) enzymes, such as reverse transcriptases, DNA polymerases, and the like; (6) various buffer mediums, e.g., hybridization and washing buffers; (7) labeled probe purification reagents and components, like spin columns, etc.; and (8) protein purification reagents; (9) signal generation and detection reagents, e.g., streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like. In particular embodiments, the kits comprise prelabeled quality controlled protein and or RNA isolated from a sample (e.g., blood or chondrocytes or synovial fluid) for use as a control.

[0554] In some embodiments, the kits are RT-PCR kits. In other embodiments, the kits are nucleic acid arrays and protein arrays. Such kits according to the subject invention will at least comprise an array having associated protein or nucleic acid members of the invention and packaging means therefore. Alternatively the protein or nucleic acid members of the invention may be prepackaged onto an array.

[0555] In some embodiments, the kits are Quantitative RT-PCR kits. In one embodiment, the quantitative RT-PCR kit includes the following: (a) primers used to amplify each of a combination of biomarkers of the invention; (b) buffers and enzymes including an reverse transcripate; (c) one or more thermos table polymerases; and (d) Sybr® Green. In a preferred embodiment, the kit of the invention also includes (a) a reference control RNA and (b) a spiked control RNA.

[0556] The invention provides kits that are useful for (a) diagnosing individuals as having arthritis, (b) differentiating between two stages of osteoarthritis (OA) and (c) diagnosing individuals as having a particular stage of osteoarthritis (OA). For example, in a particular embodiment of the invention a kit is comprised a forward and reverse primer wherein the forward and reverse primer are designed to quantitate expression of all of the species of mRNA corresponding to each of the biomarkers as identified in accordance with the invention useful in determining whether an individual has mild OA or does not have OA. In certain embodiments, at least one of the primers is designed to span an exon junction.

[0557] The invention provides kits that are useful for detecting, diagnosing, monitoring and prognosing osteoarthritis based upon the expression of protein or RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention in a sample. In certain embodiments, such kits do not include the materials and reagents for measuring the expression of a protein or RNA product of a biomarker of the invention that has been suggested by the prior art to be associated with osteoarthritis. In other embodiments, such kits include the materials and reagents for measuring the expression of a protein or RNA product of a biomarker of the invention that has been suggested by the prior art to be associated with osteoarthritis and at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45 or more genes other than the biomarkers of the invention.

[0558] The invention provides kits useful for monitoring the efficacy of one or more therapies that a subject is undergoing based upon the expression of a protein or RNA product of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the

invention in a sample. In certain embodiments, such kits do not include the materials and reagents for measuring the expression of a protein or RNA product of a biomarker of the invention that has been suggested by the prior art to be associated with osteoarthritis. In other embodiments, such kits include the materials and reagents for measuring the expression of a protein or RNA product of a biomarker of the invention that has been suggested by the prior art to be associated with osteoarthritis and at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45 or more genes other than the biomarkers of the invention.

[0559] The invention provides kits using for determining whether a subject will be responsive to a therapy based upon the expression of a protein or RNA product of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention in a sample. In certain embodiments, such kits do not include the materials and reagents for measuring the expression of a protein or RNA product of a biomarker of the invention that has been suggested by the prior art to be associated with osteoarthritis. In other embodiments, such kits include the materials and reagents for measuring the expression of a protein or RNA product of a biomarker of the invention that has been suggested by the prior art to be associated with osteoarthritis and at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45 or more genes other than the biomarkers of the invention.

[0560] The invention provides kits for measuring the expression of a RNA product of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention in a sample. In a specific embodiment, such kits comprise materials and reagents that are necessary for measuring the expression of a RNA product of a biomarker of the invention. For example, a microarray or RT-PCR kit may be produced for osteoarthritis and contain only those reagents and materials necessary for measuring the levels of RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention. Alternatively, in some embodiments, the kits can comprise materials and reagents that are not limited to those required to measure the levels of RNA products of 1,2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. For example, a microarray kit may contain reagents and materials necessary for measuring the levels of RNA products of not necessarily associated with or indicative of osteoarthritis, in addition to reagents and materials necessary for measuring the levels of the RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention. In a specific embodiment, a microarray or RT-PCR kit contains reagents and materials necessary for measuring the levels of RNA products of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, all or any combination of the biomarkers of the invention, and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 300, 350, 400, 450, or more genes other than the biomarkers of the invention, or 1-10, 1-100, 1-150, 1-200, 1-300, 1-400, 1-500, 1-1000, 25-100, 25-200, 25-300, 25-400, 25-500, 25-1000, 100-150, 100-200, 100-300, 100-400, 100-500, 100-1000, 500-1000 other genes than the biomarkers of the invention.

[0561] For nucleic acid micoarray kits, the kits generally comprise probes attached to a solid support surface. The probes may be labeled with a detectable label. In a specific embodiment, the probes are specific for an exon(s), an intron (s), an exon junction(s), or an exon-intron junction(s)), of RNA products of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. The microarray kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay. In a specific embodiment, the kits comprise instructions for diagnosing osteoarthritis. The kits may also comprise hybridization reagents and/or reagents necessary for detecting a signal produced when a probe hybridizes to a target nucleic acid sequence. Generally, the materials and reagents for the microarray kits are in one or more containers. Each component of the kit is generally in its own a suitable container.

[0562] For RT-PCR kits, the kits generally comprise preselected primers specific for particular RNA products (e.g., an exon(s), an intron(s), an exon junction(s), and an exon-intron junction(s)) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. The RT-PCR kits may also comprise enzymes suitable for reverse transcribing and/or amplifying nucleic acids (e.g., polymerases such as Taq), and deoxynucleotides and buffers needed for the reaction mixture for reverse transcription and amplification. The RT-PCR kits may also comprise probes specific for RNA products of 1,2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention. The probes may or may not be labeled with a detectable label (e.g., a fluorescent label). Each component of the RT-PCR kit is generally in its own suitable container. Thus, these kits generally comprise distinct containers suitable for each individual reagent, enzyme, primer and probe. Further, the RT-PCR kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay. In a specific embodiment, the kits contain instructions for diagnosing osteoarthritis.

[0563] In a specific embodiment, the kit is a real-time RT-PCR kit. Such a kit may comprise a 96 well plate and reagents and materials necessary for SYBR Green detection. The kit may comprise reagents and materials so that beta-actin can be used to normalize the results. The kit may also comprise controls such as water, phospate buffered saline, and phage MS2 RNA. Further, the kit may comprise instructions for performing the assay and methods for interpreting and analyzing the date resulting from the performance of the assay. In a specific embodiment, the instructions state that the level of a RNA product of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35,

40, 45, 50, all or any combination of the biomarkers of the invention should be examined at two concentrations that differ by, e.g., 5 fold to 10-fold.

[0564] For antibody based kits, the kit can comprise, for example: (1) a first antibody (which may or may not be attached to a solid support) which binds to protein of interest (e.g., a protein product of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, all or any combination of the biomarkers of the invention); and, optionally, (2) a second, different antibody which binds to either the protein, or the first antibody and is conjugated to a detectable label (e.g., a fluorescent label, radioactive isotope or enzyme). The antibody-based kits may also comprise beads for conducting an immunoprecipitation. Each component of the antibody-based kits is generally in its own suitable container. Thus, these kits generally comprise distinct containers suitable for each antibody. Further, the antibody-based kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay. In a specific embodiment, the kits contain instructions for diagnosing osteoarthritis.

6. EXAMPLES

[0565] The examples below are non-limiting and are merely representative of various aspects and features of the present invention

Example 1

Microarray Construction

[0566] An array according to one aspect of the invention was constructed as follows.

[0567] PCR products (~40 ul) of cDNA clones from OA cartilage cDNA libraries, in the same 96-well tubes used for amplification, are precipitated with 4 ul (1/10 volume) of 3M sodium acetate (pH 5.2) and 100 ul (2.5 volumes) of ethanol and stored overnight at −20° C. They are then centrifuged at 3,300 rpm at 4° C. for 1 hour. The obtained pellets were washed with 50 ul ice-cold 70% ethanol and centrifuged again for 30 minutes. The pellets are then air-dried and resuspended well in 50% dimethylsulfoxide (DMSO) or 20 ul 3×SSC overnight. The samples are then deposited either singly or in duplicate onto Gamma Amino Propyl Silane (Corning CMT-GAPS or CMT-GAP2, Catalog No. 40003, 40004) or polylysine-coated slides (Sigma Cat. No. P0425) using a robotic GMS 417 or 427 arrayer (Affymetrix, CA). The boundaries of the DNA spots on the microarray are marked with a diamond scriber. The invention provides for arrays where 10-20,000 PCR products are spotted onto a solid support to prepare an array.

[0568] The arrays are rehydrated by suspending the slides over a dish of warm particle free ddH_2O for approximately one minute (the spots will swell slightly but not run into each other) and snap-dried on a 70-80° C. inverted heating block for 3 seconds. DNA is then UV crosslinked to the slide (Stratagene, Stratalinker, 65 mJ—set display to "650" which is 650×100 uJ) or baked at 80 C for two to four hours. The arrays are placed in a slide rack. An empty slide chamber is prepared and filled with the following solution: 3.0 grams of succinic anhydride (Aldrich) is dissolved in 189 ml of 1-methyl-2-pyrrolidinone (rapid addition of reagent is crucial); immediately after the last flake of succinic anhydride dissolved, 21.0 ml of 0.2 M sodium borate is mixed in and the solution is poured into the slide chamber. The slide rack is plunged rapidly and evenly in the slide chamber and vigorously shaken up and down for a few seconds, making sure the slides never leave the solution, and then mixed on an orbital shaker for 15-20 minutes. The slide rack is then gently plunged in 95° C. ddH_2O for 2 minutes, followed by plunging five times in 95% ethanol. The slides are then air dried by allowing excess ethanol to drip onto paper towels. The arrays are then stored in the slide box at room temperature until use.

Example 2

RNA Isolation

From Whole Blood

[0569] 100 ul whole blood is obtained in a microcentrifuge tube and spun at 2,000 rpm (800 g) for 5 min at 4° C. and the supernatant removed. Pelleted cells are homogenized using TRIzol® (GIBCO/BRL) in a ratio of approximately 6 μl of TRIzol® for every 10 μl of the original blood sample and vortexed well. Samples are left for 5 minutes at room temperature. RNA is extracted using 12 μl of chloroform per 10 μl of TRIzol®. Sample is centrifuged at 12,000×g for 5 minutes at 4° C. and upper layer is collected. To upper layer, isopropanol is added in ratio of 5 μl per 10 μl of TRIzol®. Sample is left overnight at −20° C. or for one hour at −20° C. RNA is pelleted in accordance with known methods, RNA pellet air dried, and pellet resuspended in DEPC treated ddH_2O. RNA samples can also be stored in 75% ethanol where the samples are stable at room temperature for transportation.

From Centrifuged Lysed Blood

[0570] 10 ml whole blood is obtained in a Vacutainer and spun at 2,000 rpm (800 g) for 5 min at 4° C. and the plasma layer removed. Lysis Buffer is added to blood sample in a ratio of 3 parts Lysis Buffer to 1 part blood (Lysis Buffer (1 L) 0.6 g EDTA; 1.0 g $KHCO_2$, 8.2 g NH_4Cl adjusted to pH 7.4 (using NaOH)). Sample is mixed and placed on ice for 5-10 minutes until transparent. Lysed sample is centrifuged at 1000 rpm for 10 minutes at 4° C., and supernatant is aspirated. Pellet is resuspended in 5 ml Lysis Buffer, and centrifuged again at 1000 rpm for 10 minutes at 4° C. Pelleted cells are homogenized using TRIzol® (GIBCO/BRL) in a ratio of approximately 6 ml of TRIzol® for every 10 ml of the original blood sample and vortexed well. Samples are left for 5 minutes at room temperature. RNA is extracted using 1.2 ml of chloroform per 1 ml of TRIzol®. Sample is centrifuged at 12,000×g for 5 minutes at 4° C. and upper layer is collected. To upper layer, isopropanol is added in ratio of 0.5 ml per 1 ml of TRIzol®. Sample is left overnight at -20° C. or for one hour at −20° C. RNA is pelleted in accordance with known methods, RNA pellet air dried, and pellet resuspended in DEPC treated ddH_2O. RNA samples can also be stored in 75% ethanol where the samples are stable at room temperature for transportation.

From Serum Free Whole Blood

[0571] 10 ml whole blood is obtained in a Vacutainer and spun at 2,000 rpm (800 g) for 5 min at 4° C. and the plasma layer removed. Pelleted cells are homogenized using TRIzol® (GIBCO/BRL) in a ratio of approximately 6 ml of TRIzol® for every 10 ml of the original blood sample and vortexed well. Samples are left for 5 minutes at room temperature. RNA is extracted using 1.2 ml of chloroform per 1 ml of TRIzol®. Sample is centrifuged at 12,000×g for 5 minutes at 4° C. and upper layer is collected. To upper layer,

isopropanol is added in ratio of 0.5 ml per 1 ml of TRIzol®. Sample is left overnight at −20° C. or for one hour at −20° C. RNA is pelleted in accordance with known methods, RNA pellet air dried, and pellet resuspended in DEPC treated ddH_2O. RNA samples can also be stored in 75% ethanol where the samples are stable at room temperature for transportation.

Example 3

Target Nucleic Acid Preparation and Hybridization

[0572] Preparation of Fluorescent DNA Probe from mRNA

[0573] Fluorescently labeled target nucleic acid samples of RNA are prepared for analysis with an array of the invention.

[0574] 1 μg Oligo-dT primers are annealed to 10 ug of total RNA isolated from blood from patient diagnosed with osteoarthritis or suspected of having osteoarthritis in a total volume of 10 ul, by heating to 70° C. for 10 min, and cooled on ice. The mRNA is reverse transcribed by incubating the sample at 42° C. for 40 min in a 25 μl volume containing a final concentration of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 25 mM DTT, 25 mM unlabeled dNTPs, 400 units of Superscript II (200 U/uL, Gibco BRL), and 15 mM of Cy3 or Cy5 (Amersham). The reaction is stopped by the addition of 2.5 μl of 55500 mM EDTA and 5 μl of 1M NaOH, and incubation at 65° C. for 10 min. The reaction mixture is neutralized by addition of 12.5 μl of 1M TrisHCl (pH7.6).

[0575] The labeled target nucleic acid sample is purified by centrifugation in a Centricon-30 micro-concentrator (Amicon). If two different target nucleic acid samples (e.g., two samples derived from different patients) are being analyzed and compared by hybridization to the same array, each target nucleic acid sample is labeled with a different fluorescent label (e.g., Cy3 and Cy5) and separately concentrated. The separately concentrated target nucleic acid samples (Cy3 and Cy5 labeled) are combined into a fresh centricon, washed with 500 μl TE, and concentrated again to a volume of less than 7 μl. 1 μL of 10 μg/μl polyA RNA (Sigma, #P9403) and 1 μl of 10 μg/ul tRNA (Gibco-BRL, #15401-011) is added and the volume is adjusted to 9.5 μl with distilled water. For final target nucleic acid preparation 2.1 μl 20×SSC (1.5M NaCl, 150 mM NaCitrate (pH8.0)) and 0.35 μl 10%SDS is added.

Hybridization

[0576] Labeled nucleic acid is denatured by heating for 2 min at 100° C., and incubated at 37° C. for 20-30 min before being placed on a nucleic acid array under a 22 mm×22 mm glass cover slip. Hybridization is carried out at 65° C. for 14 to 18 hours in a custom slide chamber with humidity maintained by a small reservoir of 3×SSC. The array is washed by submersion and agitation for 2-5 min in 2×SSC with 0.1% SDS, followed by 1×SSC, and 0.1×SSC. Finally, the array is dried by centrifugation for 2 min in a slide rack in a Beckman GS-6 tabletop centrifuge in Microplus carriers at 650 RPM for 2 min.

Example 4

Real Time RT PCR

[0577] Real time RT PCR was performed on the genes as disclosed in Table 1 and FIGS. **1** to **4** using the SYBR® Green Kit from Qiagen (Product Number 204143). An example of the experimental results for one of the genes identified in FIGS. **1** to **4** is shown in FIG. **5**.

[0578] Either a one step (reverse transcription and PCR combined) or a two step (reverse transcription first and then subsequent PCR) can be used. In the case of the two step protocol, reverse transcription was first performed using the High-Capacity cDNA Archive Kit from Applied Biosystems (Product number 4322171) and following the protocol utilized therein.

[0579] More specifically purified RNA as described previously herein was incubated with Reverse Transcriptase buffer, dNTPs, Random primers and Reverse transcriptase and incubated for 25° C. for 10 minutes and subsequently for 37° C. for two hours and the resulting mixture utilized as the starting product for quantitative PCR.

[0580] cDNA resulting from reverse transcription was incubated with the QuantiTect SYBR® Green PCR Master Mix as provided and no adjustments were made for magnesium concentration. Uracil-N-Glycosylase was not added. 5 μM of both forward primer and reverse primer specific to the genes of the invention were added and the reaction was incubated and monitored in accordance with the standard protocol utilizing the ABI PRISM 7700/ABI GeneAmp 5700/iCycler/DNA Engine Opticon.

TABLE 7

Gene	Forward Primer	Reverse Primer	Size
B2M	GAATTCACCCCCACTGAAAA (SEQ ID NO: 49)	CCTCCATGATGCTGCTTACA (SEQ ID NO: 50)	111
G2AN	TTCCTGCTGCGTCGATTCTCA (SEQ ID NO: 51)	TTATCACCACCCGCTCAATCCA (SEQ ID NO: 52)	106
IL13RA1	CATTGTTCCAGTCATCGTCGCA (SEQ ID NO: 53)	TCTTGCCAGGATCAGGAATTGG (SEQ ID NO: 54)	101
TNFAIP6	AAGGATGGGGATTCAAGGAT (SEQ ID NO: 55)	AATTCACACACCGCCTTAGC (SEQ ID NO: 56)	133
WDR9	TTGCAGGCCCTGTTGATTTGTG (SEQ ID NO: 57)	CCAAACTAACGCAGACAGCCTC (SEQ ID NO: 58)	128
WWP2	GGCACTACACCAAGAACAGCAA (SEQ ID NO: 59)	TGCTACCGATGAGTTCGGCA (SEQ ID NO: 60)	147

TABLE 7-continued

Gene	Forward Primer	Reverse Primer	Size
BCL6	CAAGGCATTGGTGAAGACAA (SEQ ID NO: 61)	CGGCTCACAACAATGACAAC (SEQ ID NO: 62)	140
C1QR1	GCCATGGAGAACCAGTACAGTC (SEQ ID NO: 63)	GAGTTCAAAGCTCTGAGGATGGTG (SEQ ID NO: 64)	105
CCNC	CCCTTGCATGGAGGATAGTG (SEQ ID NO: 65)	CATTGCCTGGCATCTTTCTG (SEQ ID NO: 66)	127
EBNA1BP2	GCCTCCATCAGCTCAAAGTC (SEQ ID NO: 67)	TTGCACCTTCTTCCCGTATT (SEQ ID NO: 68)	179
FLJ32234	GCGGAGGATGAAGTTGTGA (SEQ ID NO: 69)	GAGTCCTTATTCAAAGTAATCGAAGG (SEQ ID NO: 70)	191
HSPCA	ATGATTGGCCAGTTCGGTGTTG (SEQ ID NO: 71)	TTCACCTGTGTCTGTCCTCACT (SEQ ID NO: 72)	147
LAMC1	CAGGCTCCATGAAGCAACAGA (SEQ ID NO: 73)	GCACTTCTCTCACTGTATGTCCCAC (SEQ ID NO: 74)	120
PAIP2	AAGATCCAAGTCGCAGCAGT (SEQ ID NO: 75)	TCCCATAACTCCTCTTTAACTTGTC (SEQ ID NO: 76)	154
ZFR	CGAGAAGAGAACATGAGGGAAGGA (SEQ ID NO: 77)	TAGAGCAGCCAGAGCGTCAA (SEQ ID NO: 78)	102
IKBKAP	GCAGCCCAGCTTAACTTTACCA (SEQ ID NO: 79)	TAATCCTGGGCACACTCTTCCA (SEQ ID NO: 80)	125
ABCA1	AGTTGGCAAGGTTGGTGAGT (SEQ ID NO: 81)	ATGGCTGTAGAGAGCTTGCGTT (SEQ ID NO 82)	111
ABCG1	TGCAGGTGGCCACTTTCGTG (SEQ ID NO: 83)	CCTTCGAACCCATACCTGACA (SEQ ID NO: 84)	139
IRF1	ACTCCAGCACTGTCGCCAT (SEQ ID NO: 85)	TGGGTGACACCTGGAAGTTGTA (SEQ ID NO: 86)	112
NCOA1	TCCAGTATCCAGGAGCAGGAA (SEQ ID NO: 87)	AGAACTCTGAGGAGGAGGGATT (SEQ ID NO 88)	110
CLIC4	TGCCCTCCCAAGTACTTAAAGC (SEQ ID NO: 89)	CAGTGCTTCATTAGCCTCTGG (SEQ ID NO: 90)	123
ACP1	TCAGAGAATTGGAGGGTAGACAGC (SEQ ID NO: 91)	TTTGGTAATCTGCCGGGCAAC (SEQ ID NO: 92)	132
ADPRT	CCCGTGACAGGCTACATGTTT (SEQ ID NO: 93)	AAGGGCAACTTCTCCCAACA (SEQ ID NO: 94)	126
ANGPTL2	AGACGTACAAGCAAGGGTTTGG (SEQ ID NO: 95)	CACCAGGAGTTTGTAGTTGCCT (SEQ ID NO: 96)	
BMPR2	AAATAGCCTGGCAGTGAGGT (SEQ ID NO: 97)	TTCAGCCATCCTTTCCTCAGCA (SEQ ID NO: 98)	106
C19orf13	CAATAGAGAACGGAGACCAACCTG (SEQ ID NO: 99)	ACCTCCTCTGCCTCTGTAT (SEQ ID NO: 100)	112
CLECSF6	ATATGCCCGTGGAAGAGACA (SEQ ID NO: 101)	TGAGCCTCCATTCTAGCACAGT (SEQ ID NO: 102)	136
CLN3	TGCCAAGCATCTACCTCGTCTT (SEQ ID NO: 103)	ATCACTGGTCTCCAGGGCGAT (SEQ ID NO: 104)	104
DNAPTP6	GGTCACAGAAGGCAACAGACTACT (SEQ ID NO: 105)	TGCCTTAGGCTTGCTTGGGTTA (SEQ ID NO: 106)	139
EFHD1	TTCCGGGAGTTCCTGCTCATTT (SEQ ID NO: 107)	AGTTCTTGGCACCTTTGACACC (SEQ ID NO: 108)	130

TABLE 7-continued

Gene	Forward Primer	Reverse Primer	Size
EGR1	GACCGCAGAGTCTTTTCCTG (SEQ ID NO: 109)	CACAAGGTGTTGCCACTGTT (SEQ ID NO: 110)	150
EXOSC10	AGTTGACTTGGAGCACCACTCT (SEQ ID NO: 111)	TTCGAAGCTCGAGGGTGTCAAT (SEQ ID NO: 112)	107
F2RL1	TGGCACCATCCAAGGAACCAAT (SEQ ID NO: 113)	TTCCAGTGAGGACAGATGCAGA (SEQ ID NO: 114)	146
FLJ11000	TGTGGTCTCTGCACCTCCTTT (SEQ ID NO: 115)	TGACCACAATCACGAGGACT (SEQ ID NO: 116)	120
FLJ11142	TCGTTTCTTGGTGACTGCTGGA (SEQ ID NO: 117)	TCCAAACCTGGGAGATGGAACT (SEQ ID NO: 118)	112
FOXK2	AGACAGCCCGAAGGATGATT (SEQ ID NO: 119)	TTGTCCGCAGTCCTGTAGTA (SEQ ID NO: 120)	150
HSPCB	CCACTTGGCAGTCAAGCACTTT (SEQ ID NO: 121)	TGATGAACACACGGCGGACATA (SEQ ID NO: 122)	146
LCMT2	TTGGCCAGTTCATGCTGCAA (SEQ ID NO: 123)	ATTCATTCATGTCCACGGCACC (SEQ ID NO: 124)	141
MAFB	CCCTTGTTTCTTTGGGTGAG (SEQ ID NO: 125)	ACGTTCTCTATGCGGTTTGG (SEQ ID NO: 126)	119
NXN	TGTAGATTCTGAGGATGACGGAG (SEQ ID NO: 127)	CCTCCTCCTCTTTGGCTTTGTACT (SEQ ID NO: 128)	101
PDCD5	ATGGCGGACGAGGAGCTTGA (SEQ ID NO: 129)	CTCATTTCTGCTTCCCTGTGCT (SEQ ID NO: 130)	119
PDK4	ACTCGGATGCTGATGAACCA (SEQ ID NO: 131)	AAGGCATCTTGGACCACTGCTA (SEQ ID NO: 132)	119
PER1	TAAGCGTAAATGTGCCTCCTCC (SEQ ID NO: 133)	TGACGGCGGATCTTTCTTGGT (SEQ ID NO: 134)	109
PF4	CCAACTGATAGCCACGCTGAAGAA (SEQ ID NO: 135)	AATGCACACACGTAGGCAGCTA (SEQ ID NO: 136)	123
PF4V1	GTTGCTGCTCCTGCCACTT (SEQ ID NO: 137)	GTGGCTATCAGTTGGGCAGT (SEQ ID NO: 138)	171
PPIF	TGCTGGAGCTGAAGGCAGAT (SEQ ID NO: 139)	TGGCACATGAAGGAAGGGAT (SEQ ID NO: 140)	127
SETBP1	TGAAGGCTTTGGAACGTACAGG (SEQ ID NO: 141)	GGGACTTGGCATCCCTGGAG (SEQ ID NO: 142)	106
SFRS6	TGGACAAACTGGATGGCACAGA (SEQ ID NO: 143)	ATCGAGACCTGGATCTGCTT (SEQ ID NO: 144)	111
SLC5A6	CCAGACCAGTTCGTCCTGTACTTT (SEQ ID NO: 145)	TATAGTGCTGAGAGAGCCGCTGAA (SEQ ID NO: 146)	105
TSPAN-2	AGGTCTGACGATCTTTGGCA (SEQ ID NO: 147)	GACAGCTCCTGTGACATTTGGT (SEQ ID NO: 148)	
YES1	ATCCAGGTATGGTGAACCGTGA (SEQ ID NO: 149)	TCAGGGTCCTTCTTCCAACACA (SEQ ID NO: 150)	124
ZNF397	GCAGCAGGTCCCAGCTAGT (SEQ ID NO: 151)	TGGAGGTGGATGTCTGTTGACT (SEQ ID NO: 152)	96
HSPCAL3	ATGATTGGCCAGTTCGGTGTTG (SEQ ID NO: 153)	TTCACCTGTGTCTGTCCTCACT (SEQ ID NO: 154)	147

Example 5

TaqMan®

[0581] Quantitative real time RT PCR was also performed using the QuantiTect™ Probe RT-PCR system from Qiagen (Product Number 204343) in conjunction with a TaqMan® dual labelled probe and primers corresponding to the gene of interest. The TaqMan® probe and primers can be ordered from Applied Biosystems Assays-On-Demand™.

[0582] The dual labelled probe contains both a fluorophore and a quencher molecule. The proximity of the fluorescent reporter with the quencher prevents the reporter from fluorescing, but during the PCR extension step, the 5'-3' exonuclease activity of the Taq DNA polymerase releases the fluorophore which allows it to fluoresce. As such, the amount of fluorescence correlates with the amount of PCR product generated.

[0583] TaqMan® quantitative PCR was performed on the TNFAIP6 gene in order to confirm the quantitative real time data obtained using SYBR® green. An example of the experimental results is showin in FIG. **6** and FIG. **8**. More specifically the TaqMan® probe was incubated with the QuantiTect PCR Master Mix, primers specific for the TNFAIP6 gene and cDNA resulting from RT PCR as described previously to a final volume of 45 ul and the PCR reaction performed using standard conditions. Results from this experiment can be seen in FIG. **6**. It would be understood by a person skilled in the art that similar results can be obtained for the other genes of the invention.

Example 6

Statistical Analysis of Real Time PCR Results

[0584] Real Time PCR analysis on blood samples isolated from individuals categorized as normal or having mild OA, moderate OA, marked OA or severe OA were statistically analyzed using known methods in order to confirm the usefulness of the genes disclosed as biomarkers.

[0585] Preferably individuals having similar age and body mass index (BMI) were selected for further analysis. Selection of samples for which comparisons could be made on the basis of age and BMI were determined using KW One Way Analysis of Variance on Ranks as would be understood by a person skilled in the art.

[0586] Delta CT value and MW Rank Sum tests were utilized on age and BMI matched sample sets of approximately 20 to 50 in size. Box plots were done on the sample sets so as to determine fold change differences as between said sets. FIGS. **5**, **7** and **8** show a representative analysis for G2AN, PER1 and ZFR respectively. As would be clear to a person skilled in the art, similar analysis can be performed for any of the sequences identified herein.

Example 7

[0587] Analysis of Gene Expression Profiles of Blood Samples from Individuals having Mild Osteoarthritis as Compared with Gene Expression Profiles from Normal Individuals using the Isolated Biomarker Described in FIG. **1**

[0588] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0589] Blood samples are taken from patients who are clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0590] Total mRNA from a drop of blood taken from each patient is first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing full length cDNA sequences for each of the 19 genes as described in FIG. **1**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 8

[0591] Analysis of Gene Expression Profiles of Blood Samples from Individuals having Moderate Osteoarthritis as Compared with Gene Expression Profiles form Normal Individuals using the Isolated Biomarker Described in FIG. **2**

[0592] This example demonstrates the use of the claimed invention to diagnose moderate osteoarthritis by detecting differential gene expression in blood samples taken from patients with moderate OA as compared to blood samples taken from healthy patients.

[0593] Blood samples are taken from patients who were clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0594] Total mRNA from a drop of blood taken from each patient is first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing full length cDNA sequences for each of the 4 genes as described in FIG. **2**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 4 genes in blood samples from patients with moderate osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 4 genes described in FIG. **2** is diagnostic for moderate osteoarthritis.

Example 9

[0595] Analysis of Gene Expression Profiles of Blood Samples form Individuals having Marked Osteoarthritis as

Compared with Gene Expression Profiles form Individuals having Moderate Osteoarthritis using the Isolated Biomarker Described in FIG. **3**

[0596] This example demonstrates the use of the claimed invention to diagnose marked osteoarthritis by detecting differential gene expression in blood samples taken from patients with marked OA as compared to blood samples taken from patients with moderate OA.

[0597] Blood samples are taken from patients who are clinically diagnosed with marked osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients who were clinically diagnosed with moderate OA. In each case, the diagnosis and staging of osteoarthritis is corroborated by a skilled Board certified physician.

[0598] Total mRNA from a drop of blood taken from each patient is first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing full length cDNA sequences for each of the 2 genes as described in FIG. **3**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 2 genes in blood samples from patients with marked osteoarthritis as compared to patients with moderate osteoarthritis is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 2 genes described in FIG. **3** is diagnostic for marked osteoarthritis.

Example 10

[0599] Analysis of Gene Expression Profiles of Blood Samples from Individuals having Severe Osteoarthritis as Compared with Gene Expression Profiles from Individuals having Marked Osteoarthritis using the Isolated Biomarker Described in FIG. **4**

[0600] This example demonstrates the use of the claimed invention to diagnose severe osteoarthritis by detecting differential gene expression in blood samples taken from patients with severe OA as compared to blood samples taken from patients with marked OA.

[0601] Blood samples are taken from patients who are clinically diagnosed with severe osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients who are clinically diagnosed with marked OA. In each case, the diagnosis and staging of osteoarthritis is corroborated by a skilled Board certified physician.

[0602] Total mRNA from a drop of blood taken from each patient is first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing full length cDNA sequences for each of the 4 genes as described in FIG. **4**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 4 genes in blood samples from patients with severe osteoarthritis as compared to patients with marked osteoarthritis is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 4 genes described in FIG. **4** is diagnostic for severe osteoarthritis.

Example 11

[0603] Analysis of Gene Expression Profiles of Blood Samples from Individuals having Osteoarthritis as Compared with Gene Expression Profiles from Healthy Individuals using the 5' Regions of the 19 Genes Described in FIG. **1**

[0604] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0605] Blood samples are taken from patients who are clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0606] Total mRNA from a drop of blood taken from each patient is first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing DNA sequences of 25 nucleotides in length corresponding to the 5' region of each of the 19 genes as described in FIG. **1**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 12

[0607] Analysis of Gene Expression Profiles of Blood Samples from Individuals having Osteoarthritis as Compared with Gene Expression Profiles from Healthy Individuals using the 3' Regions of the 19 Genes Described in FIG. **1**

[0608] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0609] Blood samples are taken from patients who were clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0610] Total mRNA from a drop of blood taken from each patient is first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing DNA sequences of 50 nucleotides in length correspond-

ing to the 3' region of each of the 19 genes as described in FIG. **1**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 13

[0611] Analysis of Gene Expression Profiles of Blood Samples from Individuals having Osteoarthritis as Compared with Gene Expression Profiles from Healthy Individuals using the Internal Coding Regions of the 19 Genes Described in FIG. **1**

[0612] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0613] Blood samples are taken from patients who are clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0614] Total mRNA from a drop of blood taken from each patient w is as first isolated using TRIzol® reagent (GIBCO) and fluorescently labeled probes for each blood sample are then generated, denatured and hybridized to a microarray containing DNA sequences of 70 nucleotides in length corresponding to the internal coding region of each of the 19 genes as described in FIG. **1**. Detection of specific hybridization to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is then determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 14

[0615] Analysis of Blood Samples from Individuals having Osteoarthritis as Compared with Blood Samples from Healthy Individuals using Monoclonal Antibodies Directed to the Polypeptides Encoded by the 19 Genes Described in FIG. **1**

[0616] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0617] Blood samples are taken from patients who are clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0618] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing, or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 Fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to full length polypeptides encoded by the 19 genes described in FIG. **1**. Detection of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 15

[0619] Analysis of Blood Samples from Individuals having Moderate Osteoarthritis as Compared with Blood Samples from Healthy Individuals using Monoclonal Antibodies Directed to the Polypeptides Encoded by the 4 Genes Described in FIG. **2**

[0620] This example demonstrates the use of the claimed invention to diagnose moderate osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0621] Blood samples are taken from patients who are clinically diagnosed with moderate osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of moderate osteoarthritis is corroborated by a skilled Board certified physician.

[0622] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing,

or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 Fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to full length polypeptides encoded by the 4 genes described in FIG. **2**. Detection of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 4 genes in blood samples from patients with moderate osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 4 genes described in FIG. **2** is diagnostic for moderate osteoarthritis. Example 16

Analysis of Blood Samples from Individuals having Marked Osteoarthritis as Compared with Blood Samples from Individuals having Moderate Arthritis using Monoclonal Antibodies Directed to the Polypeptides Encoded by the 2 Genes Described in FIG. **3**

[0623] This example demonstrates the use of the claimed invention to diagnose marked osteoarthritis by detecting differential gene expression in blood samples taken from patients with marked OA as compared to blood samples taken from patients with moderate OA.

[0624] Blood samples are taken from patients who are clinically diagnosed with marked osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients with moderate OA. In each case, the diagnosis of the stage of osteoarthritis is corroborated by a skilled Board certified physician.

[0625] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing, or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 Fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to full length polypeptides encoded by the 2 genes described in FIG. **3**. Detection of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 2 genes in blood samples from patients with marked osteoarthritis as compared to patients with moderate OA is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 2 genes described in FIG. **3** is diagnostic for marked osteoarthritis.

Example 17

[0626] Analysis of Blood Samples from Individuals having Severe Osteoarthritis as Compared with Blood Samples from Individuals having Marked Osteoarthritis using Monoclonal Antibodies Directed to the Polypeptides Encoded by the 4 Genes Described in FIG. **4**

[0627] This example demonstrates the use of the claimed invention to diagnose severe osteoarthritis by detecting differential gene expression in blood samples taken from patients with severe OA as compared to blood samples taken from patients with marked.

[0628] Blood samples are taken from patients who are clinically diagnosed with severe osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients with marked OA. In each case, the diagnosis of the stage of osteoarthritis is corroborated by a skilled Board certified physician.

[0629] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing, or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 Fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to full length polypeptides encoded by the 4 genes described in FIG. **4**. Detection of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 4 genes in blood samples from patients with severe osteoarthritis as compared to patients with marked OA is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 4 genes described in FIG. **4** is diagnostic for severe osteoarthritis.

Example 18

[0630] Analysis of Blood Samples from Individuals having Osteoarthritis as Compared with Blood Samples from Healthy Individuals using Monoclonal Antibodies Directed

to the Amino Terminal Region of Polypeptides Encoded by the 5' Regions of the 19 Genes Described in FIG. **1**

[0631] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0632] Blood samples are taken from patients who are clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0633] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing, or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 Fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to amino terminal regions of polypeptides encoded by the 5' regions of the 19 genes described in FIG. **1**. Detection of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 19

[0634] Analysis of Blood Samples from Individuals having Osteoarthritis as Compared with Blood Samples from Healthy Individuals using Monoclonal Antibodies Directed to the Carboxy Terminal Region of Polypeptides Encoded by the 3' Regions of the 19 Genes Described in FIG. **1**

[0635] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0636] Blood samples are taken from patients who were clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0637] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing, or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 Fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to the carboxy terminal regions of polypeptides encoded by the 3' regions of the 19 genes described in FIG. **1**. Detection of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 20

[0638] Analysis of Blood Samples from Individuals having Osteoarthritis as Compared with Blood Samples from Healthy Individuals using Antibodies Directed to the Internal Polypeptide Region of Polypeptides Encoded by the Internal Coding Region of the 19 Genes Described in FIG. **1**

[0639] This example demonstrates the use of the claimed invention to diagnose osteoarthritis by detecting differential gene expression in blood samples taken from patients with OA as compared to blood samples taken from healthy patients.

[0640] Blood samples are taken from patients who were clinically diagnosed with osteoarthritis as defined herein. Gene expression profiles are then analyzed and compared to profiles from patients unaffected by OA. In each case, the diagnosis of osteoarthritis is corroborated by a skilled Board certified physician.

[0641] Total cellular protein from blood taken from each patient is first isolated and labelled using the BD Clontech Protein Extraction and labelling kit (Catalogue #K1848-1 or #631786). Briefly, the Extraction Protocol consists of three main steps: mechanically disrupting the cells, solubilizing the cells, and centrifuging the extract The process may start with a cell pellet or frozen tissue and may use any method of mechanical disruption—French press, sonication, mincing, or grinding. Once disrupted, the sample is solubilized by adding the Extraction/Labeling Buffer (1:20 w/v). Because the Buffer is formulated for labeling with N-hydroxysuccinimide (NHS)-ester dyes (e.g. Cy3 and CyS dyes), it does not contain any protease inhibitors or reducing agents that would compete for reaction with the dye. After extraction, the sample is centrifuged to pellet insoluble material such as chromosomal DNA. The soluble extract is then labelled with Cy3 and Cy5 fluorescent Dyes (monofunctional NHS-esters). The labelled proteins are then incubated with an array of monoclonal antibodies which are directed to internal polypeptide regions of polypeptides encoded by the internal coding regions of the 19 genes described in FIG. **1**. Detection

of specific binding to the array is then measured by scanning with a GMS Scanner 418 and processing of the experimental data with Scanalyzer software (Michael Eisen, Stanford University), followed by GeneSpring software (Silicon Genetics, CA) analysis. Differential expression of the 19 genes in blood samples from patients with osteoarthritis as compared to healthy patients is determined by statistical analysis using the Wilcox Mann Whitney rank sum test (Glantz S A. Primer of Biostatistics. 5th ed. New York, USA: McGraw-Hill Medical Publishing Division, 2002). Differential expression of each of the 19 genes described in FIG. **1** is diagnostic for osteoarthritis.

Example 21

[0642] Application of Logistic Regression to a Subset of Nine of the Biomarkers of the Invention to Identify Combinations Useful in Differentiating a Stage of OA from Non OA.

[0643] RNA was isolated from blood samples of 259 patients with mild osteoarthritis as classified using the system of Marshall (supra) and 82 normal subjects. Primers as disclosed in Table 4 in Example 4 were used to provide data corresponding to the level of expression of the population of RNA products disclosed in Table 2. A Reference dataset consisting of ΔCt values arising from the QRT-PCR for nine of the biomarkers (EGR1, G2AN, HSPCA, IKBKAP, IL13RA1, LAMC1, MAFB, PF4, TNFAIP6) was utilized for input into logistic regression to determine the diagnostic capabilities of different combinations of ΔCt values from these 9 candidate biomarkers. Of the $2^9-1=511$ possible biomarker combinations, 254 combinations were well-behaved in maximum-likelihood logistic regression and gave significant discrimination (ROC Area>0.5) of "mild osteoarthritis" vs. "control". It is noteworthy that combinations of as few as 3 biomarkers produced ROC Areas>0.85. Table 9 below presents the logistic regression parameters for those combinations of 1, 2, 3, 4, 5, 6, 7 or 8 biomarkers giving the greatest ROC Areas for a fixed number of genes.

TABLE 9

Eqn[1]	# (genes)	T statistic	Noise Factor[2]	ROC Area	Constant	EGR1	G2AN	HSPCA	IKBKAP	IL13RA1	LAMC1	MAFB	PF4	TNFAIP6
128	1	5.8757	0.37838	0.80893	−7.7261	0	0	0	0	0	0	0	1.2613	0
MC[3]													1.2349	
384	2	6.3994	0.39773	0.82311	−11.183	0	0	0	0	0	0	0	1.2648	0.3976
MC[3]													1.2223	0.3901
192	2	6.3621	0.48444	0.82436	−4.0057	0	0	0	0	0	0	−0.6799	1.4647	0
MC[3]												−0.6664	1.4298	
208	3	7.8386	0.76278	0.87151	−12.789	0	0	0	0	1.7416	0	−1.0808	1.5045	0
MC[3]										1.6667		−1.0387	1.4276	
464	4	8.1562	0.79025	0.87818	−14.324	0	0	0	0	1.5216	0	−1.3204	1.6344	0.45691
214	5	8.3676	1.0311	0.89111	−10.806	0	−1.1191	1.8456	0	1.7009	0	−1.3922	1.5245	0
246	6	8.7395	1.0134	0.89487	−12.662	0	−1.1052	1.6446	0	1.6012	0.47022	−1.6293	1.4304	0
222	6	8.5553	1.1738	0.8957	−14.995	0	−2.2536	1.2969	1.4864	1.7789	0	−1.139	1.3704	0
223	7	8.6763	1.215	0.89779	−14.654	0.14331	−2.4461	1.2095	1.6415	1.731	0	−1.2236	1.3221	0
503	8	9.2433	1.0239	0.9028	−14.777	0.0506	−0.94879	1.6983	0	1.2055	0.45795	−1.9036	1.5243	0.50045

[0644] The six biomarker combinations with greatest overall ROC Areas (0.89-0.90) are presented in FIG. **2**, and their logistic regression parameter values are given in Table 10.

TABLE 10

Eqn[1]	# (genes)	T statistic	Noise Factor[2]	ROC Area	Constant	EGR1	G2AN	HSPCA
471	7	8.8868	1.0428	0.89487	−13.048	0.1155	−0.96585	1.8829
246	6	8.7395	1.0134	0.89487	−12.662	0	−1.1052	1.6446
247	7	8.7478	1.0145	0.89529	−12.503	2.20E−02	−1.1186	1.6405
222	6	8.5553	1.1738	0.8957	−14.995	0	−2.2536	1.2969
223	7	8.6763	1.215	0.89779	−14.654	0.14331	−2.4461	1.2095
503	8	9.2433	1.0239	0.9028	−14.777	5.06E−02	−0.94879	1.6983

Eqn[1]	IKBKAP	IL13RA1	LAMC1	MAFB	PF4	TNFAIP6
471	0	1.3037	0	−1.7394	1.6159	0.50523
246	0	1.6012	0.47022	−1.6293	1.4304	0
247	0	1.5997	0.45789	−1.6383	1.4287	0
222	1.4864	1.7789	0	−1.139	1.3704	0
223	1.6415	1.731	0	−1.2236	1.3221	0
503	0	1.2055	0.45795	−1.9036	1.5243	0.50045

[1]Equation # (out of $2^9 - 1 = 511$ possible equations) in exhaustive logistic regression analysis using LogReg v 12 m

[2]±1 σ in the logit function, propageted from an input error σ (Ct) ≈ 0.3 cycle

[3]1000 MonteCarlo samplings of exact logistic distribution, using LogXact-5

TABLE 11

Combinations of Two Genes Useful as Biomarkers of OA as Compared with Normal

	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
B2M	n/a	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
G2AN	B2M	n/a	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	G2AN		G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
1L13RA1	B2M	G2AN	n/a	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	1L13RA1	1L13RA1		1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
IKBKAP	B2M	G2AN	IL13RA1	n/a	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	IKBKAP	IKBKAP	IKBKAP		IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
TNFAIP6	B2M	G2AN	IL13RA1	IKPKAP	n/a	WDR9	WWP2	BCL6	C1QR1	CCNC
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6		TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
WDR9	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	n/a	WWP2	BCL6	C1QR1	CCNC
	WDR9	WDR9	WDR9	WDR9	WDR9		WDR9	WDR9	WDR9	WDR9
WWP2	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	n/a	BCL6	C1QR1	CCNC
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2		WWP2	WWP2	WWP2
BCL6	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	n/a	C1QR1	CCNC
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6		BCL6	BCL6
C11QR1	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	n/a	CCNC
	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1		C11QR1
CCNC	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	n/a
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	
FLJ32234	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
HSPCA	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
LAMC1	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
PAIP2	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	PAIP2	PAIP2	PAIP2	PAIP2	PAIP2	PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
IRF1	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	IRF1	IRF1	IRF1	IRF1	IRF1	IRF1	IRF1	IRF1	IRF1	IRF1
NCOA1	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1
CLIC4	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4
ABCA1	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1
ABCG1	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1

	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
TNFAIP6	FIJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
C11QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1	C11QR1
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
FLJ32234	n/a	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
HSPCA	FLJ32334	n/a	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	HSPCA		HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
LAMC1	FLJ32334	HSPCA	n/a	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	LAMC1	LAMC1		LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
PAIP2	FLJ32334	HSPCA	LAMC1	n/a	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
IRF1	FLJ32334	HSPCA	LAMC1	PAIP2	n/a	NCOA1	CLIC4	ABCA1	ABCG1
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1

TABLE 11-continued

Combinations of Two Genes Useful as Biomarkers of OA as Compared with Normal

	NCOA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	n/a	CLIC4	ABCA1	ABCG1
		NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
	CLIC4	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	n/a	ABCA1	ABCG1
		CLIC4	CLIC4	CLIC4	CLIC4	CLIC4	CLIC4		CLIC4	CLIC4
	ABCA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	n/a	ABCG1
		ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
	ABCG1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1	ABCG1

TABLE 12

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

	B2M	G2AN	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
					(a) B2M					
B2M	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
G2AN	N/A	N/A	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
			G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
			B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
1L13RA1	N/A	N/A	N/A	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
				1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
				B2M	B2M	B2M	B2M	B2M	B2M	B2M
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
					IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
					B2M	B2M	B2M	B2M	B2M	B2M
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9	WWP2	BCL6	C1QR1	CCNC
						TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
						B2M	B2M	B2M	B2M	B2M
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2	BCL6	C1QR1	CCNC
							WDR9	WDR9	WDR9	WDR9
							B2M	B2M	B2M	B2M
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6	C1QR1	CCNC
								WWP2	WWP2	WWP2
								B2M	B2M	B2M
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1	CCNC
									BCL6	BCL6
									B2M	B2M
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC
										C1QR1
										B2M
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(b) G2AN					
B2M	N/A	N/A	IL13RA1	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
			B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
			G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
G2AN	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1L13RA1	N/A	N/A	N/A	IKPKAP	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
				1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
				G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6	WDR9	WWP2	BCL6	C1QR1	CCNC
					IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
					G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9	WWP2	BCL6	C1QR1	CCNC
						TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
						G2AN	G2AN	G2AN	G2AN	G2AN
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2	BCL6	C1QR1	CCNC
							WDR9	WDR9	WDR9	WDR9
							G2AN	G2AN	G2AN	G2AN

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 G2AN	C1QR1 WWP2 G2AN	CCNC WWP2 G2AN
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 G2AN	CCNC BCL6 G2AN
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 G2AN
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(c) IL13RA1					
B2M	N/A	G2AN B2M IL13RA1	N/A	IKPKAP B2M IL13RA1	TNFAIP6 B2M IL13RA1	WDR9 B2M IL13RA1	WWP2 B2M IL13RA1	BCL6 B2M IL13RA1	C1QR1 B2M IL13RA1	CCNC B2M IL13RA1
G2AN	N/A	N/A	N/A	IKPKAP G2AN IL13RA1	TNFAIP6 G2AN IL13RA1	WDR9 G2AN IL13RA1	WWP2 G2AN IL13RA1	BCL6 G2AN IL13RA1	C1QR1 G2AN IL13RA1	CCNC G2AN IL13RA1
1L13RA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP IL13RA1	WDR9 IKBKAP IL13RA1	WWP2 IKBKAP IL13RA1	BCL6 IKBKAP IL13RA1	C1QR1 IKBKAP IL13RA1	CCNC IKBKAP IL13RA1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 IL13RA1	WWP2 TNFAIP6 IL13RA1	BCL6 TNFAIP6 IL13RA1	C1QR1 TNFAIP6 IL13RA1	CCNC TNFAIP6 IL13RA1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 IL13RA1	BCL6 WDR9 IL13RA1	C1QR1 WDR9 IL13RA1	CCNC WDR9 IL13RA1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 IL13RA1	C1QR1 WWP2 IL13RA1	CCNC WWP2 IL13RA1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 IL13RA1	CCNC BCL6 IL13RA1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 IL13RA1
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(d) IKPKAP					
B2M	N/A	G2AN B2M IKPKAP	IL13RA1 B2M IKPKAP	N/A	TNFAIP6 B2M IKPKAP	WDR9 B2M IKPKAP	WWP2 B2M IKPKAP	BCL6 B2M IKPKAP	C1QR1 B2M IKPKAP	CCNC B2M IKPKAP
G2AN	N/A	N/A	IL13RA1 G2AN IKPKAP	N/A	TNFAIP6 G2AN IKPKAP	WDR9 G2AN IKPKAP	WWP2 G2AN IKPKAP	BCL6 G2AN IKPKAP	C1QR1 G2AN IKPKAP	CCNC G2AN IKPKAP
1L13RA1	N/A	N/A	N/A	N/A	TNFAIP6 1L13RA1 IKPKAP	WDR9 1L13RA1 IKPKAP	WWP2 1L13RA1 IKPKAP	BCL6 1L13RA1 IKPKAP	C1QR1 1L13RA1 IKPKAP	CCNC 1L13RA1 IKPKAP
IKBKAP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 IKPKAP	WWP2 TNFAIP6 IKPKAP	BCL6 TNFAIP6 IKPKAP	C1QR1 TNFAIP6 IKPKAP	CCNC TNFAIP6 IKPKAP
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 IKPKAP	BCL6 WDR9 IKPKAP	C1QR1 WDR9 IKPKAP	CCNC WDR9 IKPKAP

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 IKPKAP	C1QR1 WWP2 IKPKAP	CCNC WWP2 IKPKAP
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 IKPKAP	CCNC BCL6 IKPKAP
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 IKPKAP
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(e) TNFAIP6					
B2M	N/A	G2AN B2M TNFAIP6	IL13RA1 B2M TNFAIP6	IKPKAP B2M TNFAIP6	N/A	WDR9 B2M TNFAIP6	WWP2 B2M TNFAIP6	BCL6 B2M TNFAIP6	C1QR1 B2M TNFAIP6	CCNC B2M TNFAIP6
G2AN	N/A	N/A	IL13RA1 G2AN TNFAIP6	IKPKAP G2AN TNFAIP6	N/A	WDR9 G2AN TNFAIP6	WWP2 G2AN TNFAIP6	BCL6 G2AN TNFAIP6	C1QR1 G2AN TNFAIP6	CCNC G2AN TNFAIP6
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 TNFAIP6	N/A	WDR9 1L13RA1 TNFAIP6	WWP2 1L13RA1 TNFAIP6	BCL6 1L13RA1 TNFAIP6	C1QR1 1L13RA1 TNFAIP6	CCNC 1L13RA1 TNFAIP6
IKBKAP	N/A	N/A	N/A	N/A	N/A	WDR9 IKBKAP TNFAIP6	WWP2 IKBKAP TNFAIP6	BCL6 IKBKAP TNFAIP6	C1QR1 IKBKAP TNFAIP6	CCNC IKBKAP TNFAIP6
TNFAIP6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 TNFAIP6	BCL6 WDR9 TNFAIP6	C1QR1 WDR9 TNFAIP6	CCNC WDR9 TNFAIP6
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 TNFAIP6	C1QR1 WWP2 TNFAIP6	CCNC WWP2 TNFAIP6
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 TNFAIP6	CCNC BCL6 TNFAIP6
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 TNFAIP6
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(f) WDR9					
B2M	N/A	G2AN B2M WDR9	IL13RA1 B2M WDR9	IKPKAP B2M WDR9	TNFAIP6 B2M WDR9	N/A	WWP2 B2M WDR9	BCL6 B2M WDR9	C1QR1 B2M WDR9	CCNC B2M WDR9
G2AN	N/A	N/A	IL13RA1 G2AN WDR9	IKPKAP G2AN WDR9	TNFAIP6 G2AN WDR9	N/A	WWP2 G2AN WDR9	BCL6 G2AN WDR9	C1QR1 G2AN WDR9	CCNC G2AN WDR9
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 WDR9	TNFAIP6 1L13RA1 WDR9	N/A	WWP2 1L13RA1 WDR9	BCL6 1L13RA1 WDR9	C1QR1 1L13RA1 WDR9	CCNC 1L13RA1 WDR9
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP WDR9	N/A	WWP2 IKBKAP WDR9	BCL6 IKBKAP WDR9	C1QR1 IKBKAP WDR9	CCNC IKBKAP WDR9
TNFAIP6	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 TNFAIP6 WDR9	BCL6 TNFAIP6 WDR9	C1QR1 TNFAIP6 WDR9	CCNC TNFAIP6 WDR9
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 WDR9	C1QR1 WWP2 WDR9	CCNC WWP2 WDR9
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 WDR9	CCNC BCL6 WDR9
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 WDR9
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ARCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(g) WWP2										
B2M	N/A	G2AN B2M WWP2	IL13RA1 B2M WWP2	IKPKAP B2M WWP2	TNFAIP6 B2M WWP2	WDR9 B2M WWP2	N/A	BCL6 B2M WWP2	C1QR1 B2M WWP2	CCNC B2M WWP2
G2AN	N/A	N/A	IL13RA1 G2AN WWP2	IKPKAP G2AN WWP2	TNFAIP6 G2AN WWP2	WDR9 G2AN WWP2	N/A	BCL6 G2AN WWP2	C1QR1 G2AN WWP2	CCNC G2AN WWP2
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 WWP2	TNFAIP6 1L13RA1 WWP2	WDR9 1L13RA1 WWP2	N/A	BCL6 1L13RA1 WWP2	C1QR1 1L13RA1 WWP2	CCNC 1L13RA1 WWP2
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP WWP2	WDR9 IKBKAP WWP2	N/A	BCL6 IKBKAP WWP2	C1QR1 IKBKAP WWP2	CCNC IKBKAP WWP2
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 WWP2	N/A	BCL6 TNFAIP6 WWP2	C1QR1 TNFAIP6 WWP2	CCNC TNFAIP6 WWP2
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WDR9 WWP2	C1QR1 WDR9 WWP2	CCNC WDR9 WWP2
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 WWP2	CCNC BCL6 WWP2
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 WWP2
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
(h) BCL6										
B2M	N/A	G2AN B2M BCL6	IL13RA1 B2M BCL6	IKPKAP B2M BCL6	TNFAIP6 B2M BCL6	WDR9 B2M BCL6	WWP2 B2M BCL6	N/A	C1QR1 B2M BCL6	CCNC B2M BCL6
G2AN	N/A	N/A	IL13RA1 G2AN BCL6	IKPKAP G2AN BCL6	TNFAIP6 G2AN BCL6	WDR9 G2AN BCL6	WWP2 G2AN BCL6	N/A	C1QR1 G2AN BCL6	CCNC G2AN BCL6
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 BCL6	TNFAIP6 1L13RA1 BCL6	WDR9 1L13RA1 BCL6	WWP2 1L13RA1 BCL6	N/A	C1QR1 1L13RA1 BCL6	CCNC 1L13RA1 BCL6
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP BCL6	WDR9 IKBKAP BCL6	WWP2 IKBKAP BCL6	N/A	C1QR1 IKBKAP BCL6	CCNC IKBKAP BCL6
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 BCL6	WWP2 TNFAIP6 BCL6	N/A	C1QR1 TNFAIP6 BCL6	CCNC TNFAIP6 BCL6

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 BCL6	N/A	C1QR1 WDR9 BCL6	CCNC WDR9 BCL6
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 WWP2 BCL6	CCNC WWP2 BCL6
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 BCL6
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(i) C1QR1					
B2M	N/A	G2AN B2M C1QR1	IL13RA1 B2M C1QR1	IKPKAP B2M C1QR1	TNFAIP6 B2M C1QR1	WDR9 B2M C1QR1	WWP2 B2M C1QR1	BCL6 B2M C1QR1	N/A	CCNC B2M C1QR1
G2AN	N/A	N/A	IL13RA1 G2AN C1QR1	IKPKAP G2AN C1QR1	TNFAIP6 G2AN C1QR1	WDR9 G2AN C1QR1	WWP2 G2AN C1QR1	BCL6 G2AN C1QR1	N/A	CCNC G2AN C1QR1
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 C1QR1	TNFAIP6 1L13RA1 C1QR1	WDR9 1L13RA1 C1QR1	WWP2 1L13RA1 C1QR1	BCL6 1L13RA1 C1QR1	N/A	CCNC 1L13RA1 C1QR1
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP C1QR1	WDR9 IKBKAP C1QR1	WWP2 IKBKAP C1QR1	BCL6 IKBKAP C1QR1	N/A	CCNC IKBKAP C1QR1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 C1QR1	WWP2 TNFAIP6 C1QR1	BCL6 TNFAIP6 C1QR1	N/A	CCNC TNFAIP6 C1QR1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 C1QR1	BCL6 WDR9 C1QR1	N/A	CCNC WDR9 C1QR1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 C1QR1	N/A	CCNC WWP2 C1QR1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC BCL6 C1QR1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(j) CCNC					
B2M	N/A	G2AN B2M CCNC	IL13RA1 B2M CCNC	IKPKAP B2M CCNC	TNFAIP6 B2M CCNC	WDR9 B2M CCNC	WWP2 B2M CCNC	BCL6 B2M CCNC	C1QR1 B2M CCNC	N/A
G2AN	N/A	N/A	IL13RA1 G2AN CCNC	IKPKAP G2AN CCNC	TNFAIP6 G2AN CCNC	WDR9 G2AN CCNC	WWP2 G2AN CCNC	BCL6 G2AN CCNC	C1QR1 G2AN CCNC	N/A
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 CCNC	TNFAIP6 1L13RA1 CCNC	WDR9 1L13RA1 CCNC	WWP2 1L13RA1 CCNC	BCL6 1L13RA1 CCNC	C1QR1 1L13RA1 CCNC	N/A
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP CCNC	WDR9 IKBKAP CCNC	WWP2 IKBKAP CCNC	BCL6 IKBKAP CCNC	C1QR1 IKBKAP CCNC	N/A
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 CCNC	WWP2 TNFAIP6 CCNC	BCL6 TNFAIP6 CCNC	C1QR1 TNFAIP6 CCNC	N/A

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 CCNC	BCL6 WDR9 CCNC	C1QR1 WDR9 CCNC	N/A
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 CCNC	C1QR1 WWP2 CCNC	N/A
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 CCNC	N/A
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(k) FLJ32334					
B2M	N/A	G2AN B2M FLJ32334	IL13RA1 B2M FLJ32334	IKPKAP B2M FLJ32334	TNFAIP6 B2M FLJ32334	WDR9 B2M FLJ32334	WWP2 B2M FLJ32334	BCL6 B2M FLJ32334	C1QR1 B2M FLJ32334	CCNC B2M FLJ32334
G2AN	N/A	N/A	IL13RA1 G2AN FLJ32334	IKPKAP G2AN FLJ32334	TNFAIP6 G2AN FLJ32334	WDR9 G2AN FLJ32334	WWP2 G2AN FLJ32334	BCL6 G2AN FLJ32334	C1QR1 G2AN FLJ32334	CCNC G2AN FLJ32334
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 FLJ32334	TNFAIP6 1L13RA1 FLJ32334	WDR9 1L13RA1 FLJ32334	WWP2 1L13RA1 FLJ32334	BCL6 1L13RA1 FLJ32334	C1QR1 1L13RA1 FLJ32334	CCNC 1L13RA1 FLJ32334
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP FLJ32334	WDR9 IKBKAP FLJ32334	WWP2 IKBKAP FLJ32334	BCL6 IKBKAP FLJ32334	C1QR1 IKBKAP FLJ32334	CCNC IKBKAP FLJ32334
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 FLJ32334	WWP2 TNFAIP6 FLJ32334	BCL6 TNFAIP6 FLJ32334	C1QR1 TNFAIP6 FLJ32334	CCNC TNFAIP6 FLJ32334
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 FLJ32334	BCL6 WDR9 FLJ32334	C1QR1 WDR9 FLJ32334	CCNC WDR9 FLJ32334
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 FLJ32334	C1QR1 WWP2 FLJ32334	CCNC WWP2 FLJ32334
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 FLJ32334	CCNC BCL6 FLJ32334
C11QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C11QR1 FLJ32334
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(l) HSPCA					
B2M	N/A	G2AN B2M HSPCA	IL13RA1 B2M HSPCA	IKPKAP B2M HSPCA	TNFAIP6 B2M HSPCA	WDR9 B2M HSPCA	WWP2 B2M HSPCA	BCL6 B2M HSPCA	C1QR1 B2M HSPCA	CCNC B2M HSPCA
G2AN	N/A	N/A	IL13RA1 G2AN HSPCA	IKPKAP G2AN HSPCA	TNFAIP6 G2AN HSPCA	WDR9 G2AN HSPCA	WWP2 G2AN HSPCA	BCL6 G2AN HSPCA	C1QR1 G2AN HSPCA	CCNC G2AN HSPCA
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 HSPCA	TNFAIP6 1L13RA1 HSPCA	WDR9 1L13RA1 HSPCA	WWP2 1L13RA1 HSPCA	BCL6 1L13RA1 HSPCA	C1QR1 1L13RA1 HSPCA	CCNC 1L13RA1 HSPCA
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP HSPCA	WDR9 IKBKAP HSPCA	WWP2 IKBKAP HSPCA	BCL6 IKBKAP HSPCA	C1QR1 IKBKAP HSPCA	CCNC IKBKAP HSPCA

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 HSPCA	WWP2 TNFAIP6 HSPCA	BCL6 TNFAIP6 HSPCA	C1QR1 TNFAIP6 HSPCA	CCNC TNFAIP6 HSPCA
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 HSPCA	BCL6 WDR9 HSPCA	C1QR1 WDR9 HSPCA	CCNC WDR9 HSPCA
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 HSPCA	C1QR1 WWP2 HSPCA	CCNC WWP2 HSPCA
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 HSPCA	CCNC BCL6 HSPCA
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 HSPCA
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(m) LAMC1					
B2M	N/A	G2AN B2M LAMC1	IL13RA1 B2M LAMC1	IKPKAP B2M LAMC1	TNFAIP6 B2M LAMC1	WDR9 B2M LAMC1	WWP2 B2M LAMC1	BCL6 B2M LAMC1	C1QR1 B2M LAMC1	CCNC B2M LAMC1
G2AN	N/A	N/A	IL13RA1 G2AN LAMC1	IKPKAP G2AN LAMC1	TNFAIP6 G2AN LAMC1	WDR9 G2AN LAMC1	WWP2 G2AN LAMC1	BCL6 G2AN LAMC1	C1QR1 G2AN LAMC1	CCNC G2AN LAMC1
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 LAMC1	TNFAIP6 1L13RA1 LAMC1	WDR9 1L13RA1 LAMC1	WWP2 1L13RA1 LAMC1	BCL6 1L13RA1 LAMC1	C1QR1 1L13RA1 LAMC1	CCNC 1L13RA1 LAMC1
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP LAMC1	WDR9 IKBKAP LAMC1	WWP2 IKBKAP LAMC1	BCL6 IKBKAP LAMC1	C1QR1 IKBKAP LAMC1	CCNC IKBKAP LAMC1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 LAMC1	WWP2 TNFAIP6 LAMC1	BCL6 TNFAIP6 LAMC1	C1QR1 TNFAIP6 LAMC1	CCNC TNFAIP6 LAMC1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 LAMC1	BCL6 WDR9 LAMC1	C1QR1 WDR9 LAMC1	CCNC WDR9 LAMC1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 LAMC1	C1QR1 WWP2 LAMC1	CCNC WWP2 LAMC1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 LAMC1	CCNC BCL6 LAMC1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 LAMC1
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(n) PAIP2					
B2M	N/A	G2AN B2M PAIP2	IL13RA1 B2M PAIP2	IKPKAP B2M PAIP2	TNFAIP6 B2M PAIP2	WDR9 B2M PAIP2	WWP2 B2M PAIP2	BCL6 B2M PAIP2	C1QR1 B2M PAIP2	CCNC B2M PAIP2
G2AN	N/A	N/A	IL13RA1 G2AN PAIP2	IKPKAP G2AN PAIP2	TNFAIP6 G2AN PAIP2	WDR9 G2AN PAIP2	WWP2 G2AN PAIP2	BCL6 G2AN PAIP2	C1QR1 G2AN PAIP2	CCNC G2AN PAIP2

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 PAIP2	TNFAIP6 1L13RA1 PAIP2	WDR9 1L13RA1 PAIP2	WWP2 1L13RA1 PAIP2	BCL6 1L13RA1 PAIP2	C1QR1 1L13RA1 PAIP2	CCNC 1L13RA1 PAIP2
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP PAIP2	WDR9 IKBKAP PAIP2	WWP2 IKBKAP PAIP2	BCL6 IKBKAP PAIP2	C1QR1 IKBKAP PAIP2	CCNC IKBKAP PAIP2
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 PAIP2	WWP2 TNFAIP6 PAIP2	BCL6 TNFAIP6 PAIP2	C1QR1 TNFAIP6 PAIP2	CCNC TNFAIP6 PAIP2
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 PAIP2	BCL6 WDR9 PAIP2	C1QR1 WDR9 PAIP2	CCNC WDR9 PAIP2
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 PAIP2	C1QR1 WWP2 PAIP2	CCNC WWP2 PAIP2
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 PAIP2	CCNC BCL6 PAIP2
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 PAIP2
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(o) IRF1					
B2M	N/A	G2AN B2M IRF1	IL13RA1 B2M IRF1	IKPKAP B2M IRF1	TNFAIP6 B2M IRF1	WDR9 B2M IRF1	WWP2 B2M IRF1	BCL6 B2M IRF1	C1QR1 B2M IRF1	CCNC B2M IRF1
G2AN	N/A	N/A	IL13RA1 G2AN IRF1	IKPKAP G2AN IRF1	TNFAIP6 G2AN IRF1	WDR9 G2AN IRF1	WWP2 G2AN IRF1	BCL6 G2AN IRF1	C1QR1 G2AN IRF1	CCNC G2AN IRF1
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 IRF1	TNFAIP6 1L13RA1 IRF1	WDR9 1L13RA1 IRF1	WWP2 1L13RA1 IRF1	BCL6 1L13RA1 IRF1	C1QR1 1L13RA1 IRF1	CCNC 1L13RA1 IRF1
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP IRF1	WDR9 IKBKAP IRF1	WWP2 IKBKAP IRF1	BCL6 IKBKAP IRF1	C1QR1 IKBKAP IRF1	CCNC IKBKAP IRF1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 IRF1	WWP2 TNFAIP6 IRF1	BCL6 TNFAIP6 IRF1	C1QR1 TNFAIP6 IRF1	CCNC TNFAIP6 IRF1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 IRF1	BCL6 WDR9 IRF1	C1QR1 WDR9 IRF1	CCNC WDR9 IRF1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 IRF1	C1QR1 WWP2 IRF1	CCNC WWP2 IRF1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 IRF1	CCNC BCL6 IRF1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 IRF1
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

(p) NCOA1

B2M	N/A	G2AN B2M NCOA1	IL13RA1 B2M NCOA1	IKPKAP B2M NCOA1	TNFAIP6 B2M NCOA1	WDR9 B2M NCOA1	WWP2 B2M NCOA1	BCL6 B2M NCOA1	C1QR1 B2M NCOA1	CCNC B2M NCOA1
G2AN	N/A	N/A	IL13RA1 G2AN NCOA1	IKPKAP G2AN NCOA1	TNFAIP6 G2AN NCOA1	WDR9 G2AN NCOA1	WWP2 G2AN NCOA1	BCL6 G2AN NCOA1	C1QR1 G2AN NCOA1	CCNC G2AN NCOA1
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 NCOA1	TNFAIP6 1L13RA1 NCOA1	WDR9 1L13RA1 NCOA1	WWP2 1L13RA1 NCOA1	BCL6 1L13RA1 NCOA1	C1QR1 1L13RA1 NCOA1	CCNC 1L13RA1 NCOA1
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP NCOA1	WDR9 IKBKAP NCOA1	WWP2 IKBKAP NCOA1	BCL6 IKBKAP NCOA1	C1QR1 IKBKAP NCOA1	CCNC IKBKAP NCOA1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 NCOA1	WWP2 TNFAIP6 NCOA1	BCL6 TNFAIP6 NCOA1	C1QR1 TNFAIP6 NCOA1	CCNC TNFAIP6 NCOA1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 NCOA1	BCL6 WDR9 NCOA1	C1QR1 WDR9 NCOA1	CCNC WDR9 NCOA1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 NCOA1	C1QR1 WWP2 NCOA1	CCNC WWP2 NCOA1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 NCOA1	CCNC BCL6 NCOA1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 NCOA1
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(q) CLIC4

B2M	N/A	G2AN B2M CLIC4	IL13RA1 B2M CLIC4	IKPKAP B2M CLIC4	TNFAIP6 B2M CLIC4	WDR9 B2M CLIC4	WWP2 B2M CLIC4	BCL6 B2M CLIC4	C1QR1 B2M CLIC4	CCNC B2M CLIC4
G2AN	N/A	N/A	IL13RA1 G2AN CLIC4	IKPKAP G2AN CLIC4	TNFAIP6 G2AN CLIC4	WDR9 G2AN CLIC4	WWP2 G2AN CLIC4	BCL6 G2AN CLIC4	C1QR1 G2AN CLIC4	CCNC G2AN CLIC4
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 CLIC4	TNFAIP6 1L13RA1 CLIC4	WDR9 1L13RA1 CLIC4	WWP2 1L13RA1 CLIC4	BCL6 1L13RA1 CLIC4	C1QR1 1L13RA1 CLIC4	CCNC 1L13RA1 CLIC4
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP CLIC4	WDR9 IKBKAP CLIC4	WWP2 IKBKAP CLIC4	BCL6 IKBKAP CLIC4	C1QR1 IKBKAP CLIC4	CCNC IKBKAP CLIC4
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 CLIC4	WWP2 TNFAIP6 CLIC4	BCL6 TNFAIP6 CLIC4	C1QR1 TNFAIP6 CLIC4	CCNC TNFAIP6 CLIC4
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 CLIC4	BCL6 WDR9 CLIC4	C1QR1 WDR9 CLIC4	CCNC WDR9 CLIC4
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 CLIC4	C1QR1 WWP2 CLIC4	CCNC WWP2 CLIC4
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 CLIC4	CCNC BCL6 CLIC4
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 CLIC4
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(r) ABCA1					
B2M	N/A	G2AN B2M ABCA1	IL13RA1 B2M ABCA1	IKPKAP B2M ABCA1	TNFAIP6 B2M ABCA1	WDR9 B2M ABCA1	WWP2 B2M ABCA1	BCL6 B2M ABCA1	C1QR1 B2M ABCA1	CCNC B2M ABCA1
G2AN	N/A	N/A	IL13RA1 G2AN ABCA1	IKPKAP G2AN ABCA1	TNFAIP6 G2AN ABCA1	WDR9 G2AN ABCA1	WWP2 G2AN ABCA1	BCL6 G2AN ABCA1	C1QR1 G2AN ABCA1	CCNC G2AN ABCA1
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 ABCA1	TNFAIP6 1L13RA1 ABCA1	WDR9 1L13RA1 ABCA1	WWP2 1L13RA1 ABCA1	BCL6 1L13RA1 ABCA1	C1QR1 1L13RA1 ABCA1	CCNC 1L13RA1 ABCA1
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP ABCA1	WDR9 IKBKAP ABCA1	WWP2 IKBKAP ABCA1	BCL6 IKBKAP ABCA1	C1QR1 IKBKAP ABCA1	CCNC IKBKAP ABCA1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 ABCA1	WWP2 TNFAIP6 ABCA1	BCL6 TNFAIP6 ABCA1	C1QR1 TNFAIP6 ABCA1	CCNC TNFAIP6 ABCA1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 ABCA1	BCL6 WDR9 ABCA1	C1QR1 WDR9 ABCA1	CCNC WDR9 ABCA1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 ABCA1	C1QR1 WWP2 ABCA1	CCNC WWP2 ABCA1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 ABCA1	CCNC BCL6 ABCA1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 ABCA1
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(s) ABCG1					
B2M	N/A	G2AN B2M ABCA1	IL13RA1 B2M ABCA1	IKPKAP B2M ABCA1	TNFAIP6 B2M ABCA1	WDR9 B2M ABCA1	WWP2 B2M ABCA1	BCL6 B2M ABCA1	C1QR1 B2M ABCA1	CCNC B2M ABCA1
G2AN	N/A	N/A	IL13RA1 G2AN ABCA1	IKPKAP G2AN ABCA1	TNFAIP6 G2AN ABCA1	WDR9 G2AN ABCA1	WWP2 G2AN ABCA1	BCL6 G2AN ABCA1	C1QR1 G2AN ABCA1	CCNC G2AN ABCA1
1L13RA1	N/A	N/A	N/A	IKPKAP 1L13RA1 ABCA1	TNFAIP6 1L13RA1 ABCA1	WDR9 1L13RA1 ABCA1	WWP2 1L13RA1 ABCA1	BCL6 1L13RA1 ABCA1	C1QR1 1L13RA1 ABCA1	CCNC 1L13RA1 ABCA1
IKBKAP	N/A	N/A	N/A	N/A	TNFAIP6 IKBKAP ABCA1	WDR9 IKBKAP ABCA1	WWP2 IKBKAP ABCA1	BCL6 IKBKAP ABCA1	C1QR1 IKBKAP ABCA1	CCNC IKBKAP ABCA1
TNFAIP6	N/A	N/A	N/A	N/A	N/A	WDR9 TNFAIP6 ABCA1	WWP2 TNFAIP6 ABCA1	BCL6 TNFAIP6 ABCA1	C1QR1 TNFAIP6 ABCA1	CCNC TNFAIP6 ABCA1
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	WWP2 WDR9 ABCA1	BCL6 WDR9 ABCA1	C1QR1 WDR9 ABCA1	CCNC WDR9 ABCA1
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	BCL6 WWP2 ABCA1	C1QR1 WWP2 ABCA1	CCNC WWP2 ABCA1
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C1QR1 BCL6 ABCA1	CCNC BCL6 ABCA1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	CCNC C1QR1 ABCA1
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
						(a) B2M				
B2M		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
G2AN		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
1L13RA1		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
IKBKAP		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
TNFAIP6		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
WDR9		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
WWP2		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
BCL6		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
C1QR1		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
CCNC		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
FLJ32234		N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
			B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
HSPCA		N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
				B2M	B2M	B2M	B2M	B2M	B2M	B2M
LAMC1		N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
					B2M	B2M	B2M	B2M	B2M	B2M
PAIP2		N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
						PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
						B2M	B2M	B2M	B2M	B2M
IRF1		N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
							IRF1	IRF1	IRF1	IRF1
							B2M	B2M	B2M	B2M
NCOA1		N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
								NCOA1	NCOA1	NCOA1
								B2M	B2M	B2M
CLIC4		N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
									CLIC4	CLIC4
									B2M	B2M
ABCA1		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
										ABCA1
										B2M
ABCG1		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
						(b) G2AN				
B2M		FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
		G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
G2AN		N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
						G2AN	G2AN	G2AN	G2AN	G2AN
						G2AN	G2AN	G2AN	G2AN	G2AN

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					G2AN	G2AN	G2AN	G2AN	G2AN
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						IRF1	IRF1	IRF1	IRF1
						G2AN	G2AN	G2AN	G2AN
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							G2AN	G2AN	G2AN
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								G2AN	G2AN
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									G2AN
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(c) IL13RA1

B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
1L13RA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					IL13RA1	IL13RA1	IL13RA1	IL13RA1	IL13RA1
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						IRF1	IRF1	IRF1	IRF1
						IL13RA1	IL13RA1	IL13RA1	IL13RA1
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							IL13RA1	IL13RA1	IL13RA1
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								IL13RA1	IL13RA1
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									IL13RA1
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(d) IKPKAP

B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
IKBKAP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					IKPKAP	IKPKAP	IKPKAP	IKPKAP	IKPKAP

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 IKPKAP	CLIC4 IRF1 IKPKAP	ABCA1 IRF1 IKPKAP	ABCG1 IRF1 IKPKAP
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 IKPKAP	ABCA1 NCOA1 IKPKAP	ABCG1 NCOA1 IKPKAP
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 IKPKAP	ABCG1 CLIC4 IKPKAP
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 IKPKAP
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
				(e) TNFAIP6					
B2M	FLJ32334 B2M TNFAIP6	HSPCA B2M TNFAIP6	LAMC1 B2M TNFAIP6	PAIP2 B2M TNFAIP6	IRF1 B2M TNFAIP6	NCOA1 B2M TNFAIP6	CLIC4 B2M TNFAIP6	ABCA1 B2M TNFAIP6	ABCG1 B2M TNFAIP6
G2AN	FLJ32334 G2AN TNFAIP6	HSPCA G2AN TNFAIP6	LAMC1 G2AN TNFAIP6	PAIP2 G2AN TNFAIP6	IRF1 G2AN TNFAIP6	NCOA1 G2AN TNFAIP6	CLIC4 G2AN TNFAIP6	ABCA1 G2AN TNFAIP6	ABCG1 G2AN TNFAIP6
1L13RA1	FLJ32334 1L13RA1 TNFAIP6	HSPCA 1L13RA1 TNFAIP6	LAMC1 1L13RA1 TNFAIP6	PAIP2 1L13RA1 TNFAIP6	IRF1 1L13RA1 TNFAIP6	NCOA1 1L13RA1 TNFAIP6	CLIC4 1L13RA1 TNFAIP6	ABCA1 1L13RA1 TNFAIP6	ABCG1 1L13RA1 TNFAIP6
IKBKAP	FLJ32334 IKBKAP TNFAIP6	HSPCA IKBKAP TNFAIP6	LAMC1 IKBKAP TNFAIP6	PAIP2 IKBKAP TNFAIP6	IRF1 IKBKAP TNFAIP6	NCOA1 IKBKAP TNFAIP6	CLIC4 IKBKAP TNFAIP6	ABCA1 IKBKAP TNFAIP6	ABCG1 IKBKAP TNFAIP6
TNFAIP6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
WDR9	FLJ32334 WDR9 TNFAIP6	HSPCA WDR9 TNFAIP6	LAMC1 WDR9 TNFAIP6	PAIP2 WDR9 TNFAIP6	IRF1 WDR9 TNFAIP6	NCOA1 WDR9 TNFAIP6	CLIC4 WDR9 TNFAIP6	ABCA1 WDR9 TNFAIP6	ABCG1 WDR9 TNFAIP6
WWP2	FLJ32334 WWP2 TNFAIP6	HSPCA WWP2 TNFAIP6	LAMC1 WWP2 TNFAIP6	PAIP2 WWP2 TNFAIP6	IRF1 WWP2 TNFAIP6	NCOA1 WWP2 TNFAIP6	CLIC4 WWP2 TNFAIP6	ABCA1 WWP2 TNFAIP6	ABCG1 WWP2 TNFAIP6
BCL6	FLJ32334 BCL6 TNFAIP6	HSPCA BCL6 TNFAIP6	LAMC1 BCL6 TNFAIP6	PAIP2 BCL6 TNFAIP6	IRF1 BCL6 TNFAIP6	NCOA1 BCL6 TNFAIP6	CLIC4 BCL6 TNFAIP6	ABCA1 BCL6 TNFAIP6	ABCG1 BCL6 TNFAIP6
C1QR1	FLJ32334 C1QR1 TNFAIP6	HSPCA C1QR1 TNFAIP6	LAMC1 C1QR1 TNFAIP6	PAIP2 C1QR1 TNFAIP6	IRF1 C1QR1 TNFAIP6	NCOA1 C1QR1 TNFAIP6	CLIC4 C1QR1 TNFAIP6	ABCA1 C1QR1 TNFAIP6	ABCG1 C1QR1 TNFAIP6
CCNC	FLJ32334 CCNC TNFAIP6	HSPCA CCNC TNFAIP6	LAMC1 CCNC TNFAIP6	PAIP2 CCNC TNFAIP6	IRF1 CCNC TNFAIP6	NCOA1 CCNC TNFAIP6	CLIC4 CCNC TNFAIP6	ABCA1 CCNC TNFAIP6	ABCG1 CCNC TNFAIP6
FLJ32234	N/A	HSPCA FLJ32234 TNFAIP6	LAMC1 FLJ32234 TNFAIP6	PAIP2 FLJ32234 TNFAIP6	IRF1 FLJ32234 TNFAIP6	NCOA1 FLJ32234 TNFAIP6	CLIC4 FLJ32234 TNFAIP6	ABCA1 FLJ32234 TNFAIP6	ABCG1 FLJ32234 TNFAIP6
HSPCA	N/A	N/A	LAMC1 HSPCA TNFAIP6	PAIP2 HSPCA TNFAIP6	IRF1 HSPCA TNFAIP6	NCOA1 HSPCA TNFAIP6	CLIC4 HSPCA TNFAIP6	ABCA1 HSPCA TNFAIP6	ABCG1 HSPCA TNFAIP6
LAMC1	N/A	N/A	N/A	PAIP2 LAMC1 TNFAIP6	IRF1 LAMC1 TNFAIP6	NCOA1 LAMC1 TNFAIP6	CLIC4 LAMC1 TNFAIP6	ABCA1 LAMC1 TNFAIP6	ABCG1 LAMC1 TNFAIP6
PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 TNFAIP6	NCOA1 PAIP2 TNFAIP6	CLIC4 PAIP2 TNFAIP6	ABCA1 PAIP2 TNFAIP6	ABCG1 PAIP2 TNFAIP6
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 TNFAIP6	CLIC4 IRF1 TNFAIP6	ABCA1 IRF1 TNFAIP6	ABCG1 IRF1 TNFAIP6
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 TNFAIP6	ABCA1 NCOA1 TNFAIP6	ABCG1 NCOA1 TNFAIP6
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 TNFAIP6	ABCG1 CLIC4 TNFAIP6
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 TNFAIP6
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(f) WDR9				
B2M	FLJ32334 B2M WDR9	HSPCA B2M WDR9	LAMC1 B2M WDR9	PAIP2 B2M WDR9	IRF1 B2M WDR9	NCOA1 B2M WDR9	CLIC4 B2M WDR9	ABCA1 B2M WDR9	ABCG1 B2M WDR9

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
WDR9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					WDR9	WDR9	WDR9	WDR9	WDR9
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						IRF1	IRF1	IRF1	IRF1
						WDR9	WDR9	WDR9	WDR9
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							WDR9	WDR9	WDR9
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								WDR9	WDR9
ARCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									WDR9
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(g) WWP2

B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
WWP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					WWP2	WWP2	WWP2	WWP2	WWP2
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						IRF1	IRF1	IRF1	IRF1
						WWP2	WWP2	WWP2	WWP2
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							WWP2	WWP2	WWP2
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								WWP2	WWP2
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									WWP2
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(h) BCL6				
B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ARCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
BCL6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					BCL6	BCL6	BCL6	BCL6	BCL6

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 BCL6	CLIC4 IRF1 BCL6	ABCA1 IRF1 BCL6	ABCG1 IRF1 BCL6
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 BCL6	ABCA1 NCOA1 BCL6	ABCG1 NCOA1 BCL6
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 BCL6	ABCG1 CLIC4 BCL6
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 BCL6
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(i) C1QR1				
B2M	FLJ32334 B2M C1QR1	HSPCA B2M C1QR1	LAMC1 B2M C1QR1	PAIP2 B2M C1QR1	IRF1 B2M C1QR1	NCOA1 B2M C1QR1	CLIC4 B2M C1QR1	ABCA1 B2M C1QR1	ABCG1 B2M C1QR1
G2AN	FLJ32334 G2AN C1QR1	HSPCA G2AN C1QR1	LAMC1 G2AN C1QR1	PAIP2 G2AN C1QR1	IRF1 G2AN C1QR1	NCOA1 G2AN C1QR1	CLIC4 G2AN C1QR1	ABCA1 G2AN C1QR1	ABCG1 G2AN C1QR1
1L13RA1	FLJ32334 1L13RA1 C1QR1	HSPCA 1L13RA1 C1QR1	LAMC1 1L13RA1 C1QR1	PAIP2 1L13RA1 C1QR1	IRF1 1L13RA1 C1QR1	NCOA1 1L13RA1 C1QR1	CLIC4 1L13RA1 C1QR1	ABCA1 1L13RA1 C1QR1	ABCG1 1L13RA1 C1QR1
IKBKAP	FLJ32334 IKBKAP C1QR1	HSPCA IKBKAP C1QR1	LAMC1 IKBKAP C1QR1	PAIP2 IKBKAP C1QR1	IRF1 IKBKAP C1QR1	NCOA1 IKBKAP C1QR1	CLIC4 IKBKAP C1QR1	ABCA1 IKBKAP C1QR1	ABCG1 IKBKAP C1QR1
TNFAIP6	FLJ32334 TNFAIP6 C1QR1	HSPCA TNFAIP6 C1QR1	LAMC1 TNFAIP6 C1QR1	PAIP2 TNFAIP6 C1QR1	IRF1 TNFAIP6 C1QR1	NCOA1 TNFAIP6 C1QR1	CLIC4 TNFAIP6 C1QR1	ABCA1 TNFAIP6 C1QR1	ABCG1 TNFAIP6 C1QR1
WDR9	FLJ32334 WDR9 C1QR1	HSPCA WDR9 C1QR1	LAMC1 WDR9 C1QR1	PAIP2 WDR9 C1QR1	IRF1 WDR9 C1QR1	NCOA1 WDR9 C1QR1	CLIC4 WDR9 C1QR1	ABCA1 WDR9 C1QR1	ABCG1 WDR9 C1QR1
WWP2	FLJ32334 WWP2 C1QR1	HSPCA WWP2 C1QR1	LAMC1 WWP2 C1QR1	PAIP2 WWP2 C1QR1	IRF1 WWP2 C1QR1	NCOA1 WWP2 C1QR1	CLIC4 WWP2 C1QR1	ABCA1 WWP2 C1QR1	ABCG1 WWP2 C1QR1
BCL6	FLJ32334 BCL6 C1QR1	HSPCA BCL6 C1QR1	LAMC1 BCL6 C1QR1	PAIP2 BCL6 C1QR1	IRF1 BCL6 C1QR1	NCOA1 BCL6 C1QR1	CLIC4 BCL6 C1QR1	ABCA1 BCL6 C1QR1	ABCG1 BCL6 C1QR1
C1QR1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CCNC	FLJ32334 CCNC C1QR1	HSPCA CCNC C1QR1	LAMC1 CCNC C1QR1	PAIP2 CCNC C1QR1	IRF1 CCNC C1QR1	NCOA1 CCNC C1QR1	CLIC4 CCNC C1QR1	ABCA1 CCNC C1QR1	ABCG1 CCNC C1QR1
FLJ32234	N/A	HSPCA FLJ32234 C1QR1	LAMC1 FLJ32234 C1QR1	PAIP2 FLJ32234 C1QR1	IRF1 FLJ32234 C1QR1	NCOA1 FLJ32234 C1QR1	CLIC4 FLJ32234 C1QR1	ABCA1 FLJ32234 C1QR1	ABCG1 FLJ32234 C1QR1
HSPCA	N/A	N/A	LAMC1 HSPCA C1QR1	PAIP2 HSPCA C1QR1	IRF1 HSPCA C1QR1	NCOA1 HSPCA C1QR1	CLIC4 HSPCA C1QR1	ABCA1 HSPCA C1QR1	ABCG1 HSPCA C1QR1
LAMC1	N/A	N/A	N/A	PAIP2 LAMC1 C1QR1	IRF1 LAMC1 C1QR1	NCOA1 LAMC1 C1QR1	CLIC4 LAMC1 C1QR1	ABCA1 LAMC1 C1QR1	ABCG1 LAMC1 C1QR1
PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 C1QR1	NCOA1 PAIP2 C1QR1	CLIC4 PAIP2 C1QR1	ABCA1 PAIP2 C1QR1	ABCG1 PAIP2 C1QR1
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 C1QR1	CLIC4 IRF1 C1QR1	ABCA1 IRF1 C1QR1	ABCG1 IRF1 C1QR1
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 C1QR1	ABCA1 NCOA1 C1QR1	ABCG1 NCOA1 C1QR1
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 C1QR1	ABCG1 CLIC4 C1QR1
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 C1QR1
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(j) CCNC				
B2M	FLJ32334 B2M CCNC	HSPCA B2M CCNC	LAMC1 B2M CCNC	PAIP2 B2M CCNC	IRF1 B2M CCNC	NCOA1 B2M CCNC	CLIC4 B2M CCNC	ABCA1 B2M CCNC	ABCG1 B2M CCNC

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
CCNC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
				CCNC	CCNC	CCNC	CCNC	CCNC	CCNC
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
					CCNC	CCNC	CCNC	CCNC	CCNC
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						IRF1	IRF1	IRF1	IRF1
						CCNC	CCNC	CCNC	CCNC
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							CCNC	CCNC	CCNC
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								CCNC	CCNC
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									CCNC
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(k) FLJ32334

B2M	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334
G2AN	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334
1L13RA1	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334
IKBKAP	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334
TNFAIP6	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334
WDR9	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334
WWP2	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2
		FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334	FLJ32334

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

BCL6	N/A	HSPCA BCL6 FLJ32334	LAMC1 BCL6 FLJ32334	PAIP2 BCL6 FLJ32334	IRF1 BCL6 FLJ32334	NCOA1 BCL6 FLJ32334	CLIC4 BCL6 FLJ32334	ABCA1 BCL6 FLJ32334	ABCG1 BCL6 FLJ32334
C11QR1	N/A	HSPCA C11QR1 FLJ32334	LAMC1 C11QR1 FLJ32334	PAIP2 C11QR1 FLJ32334	IRF1 C11QR1 FLJ32334	NCOA1 C11QR1 FLJ32334	CLIC4 C11QR1 FLJ32334	ABCA1 C11QR1 FLJ32334	ABCG1 C11QR1 FLJ32334
CCNC	N/A	HSPCA CCNC FLJ32334	LAMC1 CCNC FLJ32334	PAIP2 CCNC FLJ32334	IRF1 CCNC FLJ32334	NCOA1 CCNC FLJ32334	CLIC4 CCNC FLJ32334	ABCA1 CCNC FLJ32334	ABCG1 CCNC FLJ32334
FLJ32234	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HSPCA	N/A	N/A	LAMC1 HSPCA FLJ32334	PAIP2 HSPCA FLJ32334	IRF1 HSPCA FLJ32334	NCOA1 HSPCA FLJ32334	CLIC4 HSPCA FLJ32334	ABCA1 HSPCA FLJ32334	ABCG1 HSPCA FLJ32334
LAMC1	N/A	N/A	N/A	PAIP2 LAMC1 FLJ32334	IRF1 LAMC1 FLJ32334	NCOA1 LAMC1 FLJ32334	CLIC4 LAMC1 FLJ32334	ABCA1 LAMC1 FLJ32334	ABCG1 LAMC1 FLJ32334
PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 FLJ32334	NCOA1 PAIP2 FLJ32334	CLIC4 PAIP2 FLJ32334	ABCA1 PAIP2 FLJ32334	ABCG1 PAIP2 FLJ32334
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 FLJ32334	CLIC4 IRF1 FLJ32334	ABCA1 IRF1 FLJ32334	ABCG1 IRF1 FLJ32334
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 FLJ32334	ABCA1 NCOA1 FLJ32334	ABCG1 NCOA1 FLJ32334
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 FLJ32334	ABCG1 CLIC4 FLJ32334
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 FLJ32334
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(l) HSPCA				
B2M	FLJ32334 B2M HSPCA	N/A	LAMC1 B2M HSPCA	PAIP2 B2M HSPCA	IRF1 B2M HSPCA	NCOA1 B2M HSPCA	CLIC4 B2M HSPCA	ABCA1 B2M HSPCA	ABCG1 B2M HSPCA
G2AN	FLJ32334 G2AN HSPCA	N/A	LAMC1 G2AN HSPCA	PAIP2 G2AN HSPCA	IRF1 G2AN HSPCA	NCOA1 G2AN HSPCA	CLIC4 G2AN HSPCA	ABCA1 G2AN HSPCA	ABCG1 G2AN HSPCA
1L13RA1	FLJ32334 1L13RA1 HSPCA	N/A	LAMC1 1L13RA1 HSPCA	PAIP2 1L13RA1 HSPCA	IRF1 1L13RA1 HSPCA	NCOA1 1L13RA1 HSPCA	CLIC4 1L13RA1 HSPCA	ABCA1 1L13RA1 HSPCA	ABCG1 1L13RA1 HSPCA
IKBKAP	FLJ32334 IKBKAP HSPCA	N/A	LAMC1 IKBKAP HSPCA	PAIP2 IKBKAP HSPCA	IRF1 IKBKAP HSPCA	NCOA1 IKBKAP HSPCA	CLIC4 IKBKAP HSPCA	ABCA1 IKBKAP HSPCA	ABCG1 IKBKAP HSPCA
TNFAIP6	FLJ32334 TNFAIP6 HSPCA	N/A	LAMC1 TNFAIP6 HSPCA	PAIP2 TNFAIP6 HSPCA	IRF1 TNFAIP6 HSPCA	NCOA1 TNFAIP6 HSPCA	CLIC4 TNFAIP6 HSPCA	ABCA1 TNFAIP6 HSPCA	ABCG1 TNFAIP6 HSPCA
WDR9	FLJ32334 WDR9 HSPCA	N/A	LAMC1 WDR9 HSPCA	PAIP2 WDR9 HSPCA	IRF1 WDR9 HSPCA	NCOA1 WDR9 HSPCA	CLIC4 WDR9 HSPCA	ABCA1 WDR9 HSPCA	ABCG1 WDR9 HSPCA
WWP2	FLJ32334 WWP2 HSPCA	N/A	LAMC1 WWP2 HSPCA	PAIP2 WWP2 HSPCA	IRF1 WWP2 HSPCA	NCOA1 WWP2 HSPCA	CLIC4 WWP2 HSPCA	ABCA1 WWP2 HSPCA	ABCG1 WWP2 HSPCA
BCL6	FLJ32334 BCL6 HSPCA	N/A	LAMC1 BCL6 HSPCA	PAIP2 BCL6 HSPCA	IRF1 BCL6 HSPCA	NCOA1 BCL6 HSPCA	CLIC4 BCL6 HSPCA	ABCA1 BCL6 HSPCA	ABCG1 BCL6 HSPCA
C1QR1	FLJ32334 C1QR1 HSPCA	N/A	LAMC1 C1QR1 HSPCA	PAIP2 C1QR1 HSPCA	IRF1 C1QR1 HSPCA	NCOA1 C1QR1 HSPCA	CLIC4 C1QR1 HSPCA	ABCA1 C1QR1 HSPCA	ABCG1 C1QR1 HSPCA
CCNC	FLJ32334 CCNC HSPCA	N/A	LAMC1 CCNC HSPCA	PAIP2 CCNC HSPCA	IRF1 CCNC HSPCA	NCOA1 CCNC HSPCA	CLIC4 CCNC HSPCA	ABCA1 CCNC HSPCA	ABCG1 CCNC HSPCA
FLJ32234	N/A	N/A	LAMC1 FLJ32234 HSPCA	PAIP2 FLJ32234 HSPCA	IRF1 FLJ32234 HSPCA	NCOA1 FLJ32234 HSPCA	CLIC4 FLJ32234 HSPCA	ABCA1 FLJ32234 HSPCA	ABCG1 FLJ32234 HSPCA
HSPCA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LAMC1	N/A	N/A	N/A	PAIP2 LAMC1 HSPCA	IRF1 LAMC1 HSPCA	NCOA1 LAMC1 HSPCA	CLIC4 LAMC1 HSPCA	ABCA1 LAMC1 HSPCA	ABCG1 LAMC1 HSPCA
PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 HSPCA	NCOA1 PAIP2 HSPCA	CLIC4 PAIP2 HSPCA	ABCA1 PAIP2 HSPCA	ABCG1 PAIP2 HSPCA

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 HSPCA	CLIC4 IRF1 HSPCA	ABCA1 IRF1 HSPCA	ABCG1 IRF1 HSPCA
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 HSPCA	ABCA1 NCOA1 HSPCA	ABCG1 NCOA1 HSPCA
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 HSPCA	ABCG1 CLIC4 HSPCA
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 HSPCA
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(m) LAMC1				
B2M	FLJ32334 B2M LAMC1	HSPCA B2M LAMC1	N/A	PAIP2 B2M LAMC1	IRF1 B2M LAMC1	NCOA1 B2M LAMC1	CLIC4 B2M LAMC1	ABCA1 B2M LAMC1	ABCG1 B2M LAMC1
G2AN	FLJ32334 G2AN LAMC1	HSPCA G2AN LAMC1	N/A	PAIP2 G2AN LAMC1	IRF1 G2AN LAMC1	NCOA1 G2AN LAMC1	CLIC4 G2AN LAMC1	ABCA1 G2AN LAMC1	ABCG1 G2AN LAMC1
1L13RA1	FLJ32334 1L13RA1 LAMC1	HSPCA 1L13RA1 LAMC1	N/A	PAIP2 1L13RA1 LAMC1	IRF1 1L13RA1 LAMC1	NCOA1 1L13RA1 LAMC1	CLIC4 1L13RA1 LAMC1	ABCA1 1L13RA1 LAMC1	ABCG1 1L13RA1 LAMC1
IKBKAP	FLJ32334 IKBKAP LAMC1	HSPCA IKBKAP LAMC1	N/A	PAIP2 IKBKAP LAMC1	IRF1 IKBKAP LAMC1	NCOA1 IKBKAP LAMC1	CLIC4 IKBKAP LAMC1	ABCA1 IKBKAP LAMC1	ABCG1 IKBKAP LAMC1
TNFAIP6	FLJ32334 TNFAIP6 LAMC1	HSPCA TNFAIP6 LAMC1	N/A	PAIP2 TNFAIP6 LAMC1	IRF1 TNFAIP6 LAMC1	NCOA1 TNFAIP6 LAMC1	CLIC4 TNFAIP6 LAMC1	ABCA1 TNFAIP6 LAMC1	ABCG1 TNFAIP6 LAMC1
WDR9	FLJ32334 WDR9 LAMC1	HSPCA WDR9 LAMC1	N/A	PAIP2 WDR9 LAMC1	IRF1 WDR9 LAMC1	NCOA1 WDR9 LAMC1	CLIC4 WDR9 LAMC1	ABCA1 WDR9 LAMC1	ABCG1 WDR9 LAMC1
WWP2	FLJ32334 WWP2 LAMC1	HSPCA WWP2 LAMC1	N/A	PAIP2 WWP2 LAMC1	IRF1 WWP2 LAMC1	NCOA1 WWP2 LAMC1	CLIC4 WWP2 LAMC1	ABCA1 WWP2 LAMC1	ABCG1 WWP2 LAMC1
BCL6	FLJ32334 BCL6 LAMC1	HSPCA BCL6 LAMC1	N/A	PAIP2 BCL6 LAMC1	IRF1 BCL6 LAMC1	NCOA1 BCL6 LAMC1	CLIC4 BCL6 LAMC1	ABCA1 BCL6 LAMC1	ABCG1 BCL6 LAMC1
C1QR1	FLJ32334 C1QR1 LAMC1	HSPCA C1QR1 LAMC1	N/A	PAIP2 C1QR1 LAMC1	IRF1 C1QR1 LAMC1	NCOA1 C1QR1 LAMC1	CLIC4 C1QR1 LAMC1	ABCA1 C1QR1 LAMC1	ABCG1 C1QR1 LAMC1
CCNC	FLJ32334 CCNC LAMC1	HSPCA CCNC LAMC1	N/A	PAIP2 CCNC LAMC1	IRF1 CCNC LAMC1	NCOA1 CCNC LAMC1	CLIC4 CCNC LAMC1	ABCA1 CCNC LAMC1	ABCG1 CCNC LAMC1
FLJ32234	N/A	HSPCA FLJ32234 LAMC1	N/A	PAIP2 FLJ32234 LAMC1	IRF1 FLJ32234 LAMC1	NCOA1 FLJ32234 LAMC1	CLIC4 FLJ32234 LAMC1	ABCA1 FLJ32234 LAMC1	ABCG1 FLJ32234 LAMC1
HSPCA	N/A	N/A	N/A	PAIP2 HSPCA LAMC1	IRF1 HSPCA LAMC1	NCOA1 HSPCA LAMC1	CLIC4 HSPCA LAMC1	ABCA1 HSPCA LAMC1	ABCG1 HSPCA LAMC1
LAMC1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 LAMC1	NCOA1 PAIP2 LAMC1	CLIC4 PAIP2 LAMC1	ABCA1 PAIP2 LAMC1	ABCG1 PAIP2 LAMC1
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 LAMC1	CLIC4 IRF1 LAMC1	ABCA1 IRF1 LAMC1	ABCG1 IRF1 LAMC1
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 NCOA1 LAMC1	ABCA1 NCOA1 LAMC1	ABCG1 NCOA1 LAMC1
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 LAMC1	ABCG1 CLIC4 LAMC1
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 LAMC1
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(n) PAIP2				
B2M	FLJ32334 B2M PAIP2	HSPCA B2M PAIP2	LAMC1 B2M PAIP2	N/A	IRF1 B2M PAIP2	NCOA1 B2M PAIP2	CLIC4 B2M PAIP2	ABCA1 B2M PAIP2	ABCG1 B2M PAIP2

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

G2AN	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN		G2AN	G2AN	G2AN	G2AN	G2AN
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
1L13RA1	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1		1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
IKBKAP	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP		IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
TNFAIP6	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6		TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
WDR9	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9		WDR9	WDR9	WDR9	WDR9	WDR9
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
WWP2	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2		WWP2	WWP2	WWP2	WWP2	WWP2
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
BCL6	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6		BCL6	BCL6	BCL6	BCL6	BCL6
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
C1QR1	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1		C1QR1	C1QR1	C1QR1	C1QR1	C1QR1
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
CCNC	FLJ32334	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC		CCNC	CCNC	CCNC	CCNC	CCNC
	PAIP2	PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
FLJ32234	N/A	HSPCA	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234
		PAIP2	PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
HSPCA	N/A	N/A	LAMC1	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA		HSPCA	HSPCA	HSPCA	HSPCA	HSPCA
			PAIP2		PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
LAMC1	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	ABCG1
					LAMC1	LAMC1	LAMC1	LAMC1	LAMC1
					PAIP2	PAIP2	PAIP2	PAIP2	PAIP2
PAIP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						IRF1	IRF1	IRF1	IRF1
						PAIP2	PAIP2	PAIP2	PAIP2
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							PAIP2	PAIP2	PAIP2
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								PAIP2	PAIP2
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									PAIP2
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(o) IRF1				
B2M	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M		B2M	B2M	B2M	B2M
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN		G2AN	G2AN	G2AN	G2AN
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1		1L13RA1	1L13RA1	1L13RA1	1L13RA1
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP		IKBKAP	IKBKAP	IKBKAP	IKBKAP
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6		TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9		WDR9	WDR9	WDR9	WDR9
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2		WWP2	WWP2	WWP2	WWP2
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6		BCL6	BCL6	BCL6	BCL6
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1		C1QR1	C1QR1	C1QR1	C1QR1
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC		CCNC	CCNC	CCNC	CCNC
	IRF1	IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234		FLJ32234	FLJ32234	FLJ32234	FLJ32234
		IRF1	IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
HSPCA	N/A	N/A	LAMC1	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA		HSPCA	HSPCA	HSPCA	HSPCA
			IRF1	IRF1		IRF1	IRF1	IRF1	IRF1
LAMC1	N/A	N/A	N/A	PAIP2	N/A	NCOA1	CLIC4	ABCA1	ABCG1
				LAMC1		LAMC1	LAMC1	LAMC1	LAMC1
				IRF1		IRF1	IRF1	IRF1	IRF1
PAIP2	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	ABCG1
						PAIP2	PAIP2	PAIP2	PAIP2
						IRF1	IRF1	IRF1	IRF1
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	ABCG1
							NCOA1	NCOA1	NCOA1
							IRF1	IRF1	IRF1
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	ABCG1
								CLIC4	CLIC4
								IRF1	IRF1
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									IRF1
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(p) NCOA1				
B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	B2M	B2M	B2M	B2M	B2M		B2M	B2M	B2M
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN		G2AN	G2AN	G2AN
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1		1L13RA1	1L13RA1	1L13RA1
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP		IKBKAP	IKBKAP	IKBKAP
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6		TNFAIP6	TNFAIP6	TNFAIP6
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9		WDR9	WDR9	WDR9
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2		WWP2	WWP2	WWP2
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6		BCL6	BCL6	BCL6
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1		C1QR1	C1QR1	C1QR1
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC		CCNC	CCNC	CCNC
	NCOA1	NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234		FLJ32234	FLJ32234	FLJ32234
		NCOA1	NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
			HSPCA	HSPCA	HSPCA		HSPCA	HSPCA	HSPCA
			NCOA1	NCOA1	NCOA1		NCOA1	NCOA1	NCOA1
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	N/A	CLIC4	ABCA1	ABCG1
				LAMC1	LAMC1		LAMC1	LAMC1	LAMC1
				NCOA1	NCOA1		NCOA1	NCOA1	NCOA1

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 NCOA1	N/A	CLIC4 PAIP2 NCOA1	ABCA1 PAIP2 NCOA1	ABCG1 PAIP2 NCOA1
IRF1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4 IRF1 NCOA1	ABCA1 IRF1 NCOA1	ABCG1 IRF1 NCOA1
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 CLIC4 NCOA1	ABCG1 CLIC4 NCOA1
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 NCOA1
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(q) CLIC4				
B2M	FLJ32334 B2M CLIC4	HSPCA B2M CLIC4	LAMC1 B2M CLIC4	PAIP2 B2M CLIC4	IRF1 B2M CLIC4	NCOA1 B2M CLIC4	N/A	ABCA1 B2M CLIC4	ABCG1 B2M CLIC4
G2AN	FLJ32334 G2AN CLIC4	HSPCA G2AN CLIC4	LAMC1 G2AN CLIC4	PAIP2 G2AN CLIC4	IRF1 G2AN CLIC4	NCOA1 G2AN CLIC4	N/A	ABCA1 G2AN CLIC4	ABCG1 G2AN CLIC4
1L13RA1	FLJ32334 1L13RA1 CLIC4	HSPCA 1L13RA1 CLIC4	LAMC1 1L13RA1 CLIC4	PAIP2 1L13RA1 CLIC4	IRF1 1L13RA1 CLIC4	NCOA1 1L13RA1 CLIC4	N/A	ABCA1 1L13RA1 CLIC4	ABCG1 1L13RA1 CLIC4
IKBKAP	FLJ32334 IKBKAP CLIC4	HSPCA IKBKAP CLIC4	LAMC1 IKBKAP CLIC4	PAIP2 IKBKAP CLIC4	IRF1 IKBKAP CLIC4	NCOA1 IKBKAP CLIC4	N/A	ABCA1 IKBKAP CLIC4	ABCG1 IKBKAP CLIC4
TNFAIP6	FLJ32334 TNFAIP6 CLIC4	HSPCA TNFAIP6 CLIC4	LAMC1 TNFAIP6 CLIC4	PAIP2 TNFAIP6 CLIC4	IRF1 TNFAIP6 CLIC4	NCOA1 TNFAIP6 CLIC4	N/A	ABCA1 TNFAIP6 CLIC4	ABCG1 TNFAIP6 CLIC4
WDR9	FLJ32334 WDR9 CLIC4	HSPCA WDR9 CLIC4	LAMC1 WDR9 CLIC4	PAIP2 WDR9 CLIC4	IRF1 WDR9 CLIC4	NCOA1 WDR9 CLIC4	N/A	ABCA1 WDR9 CLIC4	ABCG1 WDR9 CLIC4
WWP2	FLJ32334 WWP2 CLIC4	HSPCA WWP2 CLIC4	LAMC1 WWP2 CLIC4	PAIP2 WWP2 CLIC4	IRF1 WWP2 CLIC4	NCOA1 WWP2 CLIC4	N/A	ABCA1 WWP2 CLIC4	ABCG1 WWP2 CLIC4
BCL6	FLJ32334 BCL6 CLIC4	HSPCA BCL6 CLIC4	LAMC1 BCL6 CLIC4	PAIP2 BCL6 CLIC4	IRF1 BCL6 CLIC4	NCOA1 BCL6 CLIC4	N/A	ABCA1 BCL6 CLIC4	ABCG1 BCL6 CLIC4
C1QR1	FLJ32334 C1QR1 CLIC4	HSPCA C1QR1 CLIC4	LAMC1 C1QR1 CLIC4	PAIP2 C1QR1 CLIC4	IRF1 C1QR1 CLIC4	NCOA1 C1QR1 CLIC4	N/A	ABCA1 C1QR1 CLIC4	ABCG1 C1QR1 CLIC4
CCNC	FLJ32334 CCNC CLIC4	HSPCA CCNC CLIC4	LAMC1 CCNC CLIC4	PAIP2 CCNC CLIC4	IRF1 CCNC CLIC4	NCOA1 CCNC CLIC4	N/A	ABCA1 CCNC CLIC4	ABCG1 CCNC CLIC4
FLJ32234	N/A	HSPCA FLJ32234 CLIC4	LAMC1 FLJ32234 CLIC4	PAIP2 FLJ32234 CLIC4	IRF1 FLJ32234 CLIC4	NCOA1 FLJ32234 CLIC4	N/A	ABCA1 FLJ32234 CLIC4	ABCG1 FLJ32234 CLIC4
HSPCA	N/A	N/A	LAMC1 HSPCA CLIC4	PAIP2 HSPCA CLIC4	IRF1 HSPCA CLIC4	NCOA1 HSPCA CLIC4	N/A	ABCA1 HSPCA CLIC4	ABCG1 HSPCA CLIC4
LAMC1	N/A	N/A	N/A	PAIP2 LAMC1 CLIC4	IRF1 LAMC1 CLIC4	NCOA1 LAMC1 CLIC4	N/A	ABCA1 LAMC1 CLIC4	ABCG1 LAMC1 CLIC4
PAIP2	N/A	N/A	N/A	N/A	IRF1 PAIP2 CLIC4	NCOA1 PAIP2 CLIC4	N/A	ABCA1 PAIP2 CLIC4	ABCG1 PAIP2 CLIC4
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1 IRF1 CLIC4	N/A	ABCA1 IRF1 CLIC4	ABCG1 IRF1 CLIC4
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1 NCOA1 CLIC4	ABCG1 NCOA1 CLIC4
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1 ABCA1 CLIC4
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(r) ABCA1				
B2M	FLJ32334 B2M ABCA1	HSPCA B2M ABCA1	LAMC1 B2M ABCA1	PAIP2 B2M ABCA1	IRF1 B2M ABCA1	NCOA1 B2M ABCA1	CLIC4 B2M ABCA1	N/A	ABCG1 B2M ABCA1

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN		G2AN
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1		1L13RA1
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP		IKBKAP
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6		TNFAIP6
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9		WDR9
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2		WWP2
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6		BCL6
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1		C1QR1
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC		CCNC
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234		FLJ32234
		ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA		HSPCA
			ABCA1	ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	N/A	ABCG1
				LAMC1	LAMC1	LAMC1	LAMC1		LAMC1
				ABCA1	ABCA1	ABCA1	ABCA1		ABCA1
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	N/A	ABCG1
					PAIP2	PAIP2	PAIP2		PAIP2
					ABCA1	ABCA1	ABCA1		ABCA1
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	N/A	ABCG1
						IRF1	IRF1		IRF1
						ABCA1	ABCA1		ABCA1
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									CLIC4
									ABCA1
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCG1
									ABCA1
									ABCA1
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					(s) ABCG1				
B2M	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	B2M	B2M	B2M	B2M	B2M	B2M	B2M	B2M	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
G2AN	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	G2AN	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
1L13RA1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	1L13RA1	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
IKBKAP	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	IKBKAP	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
TNFAIP6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	TNFAIP6	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
WDR9	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	WDR9	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	

TABLE 12-continued

Combinations of Three Genes Useful as Biomarkers of OA as Compared with Normal

WWP2	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	WWP2	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
BCL6	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	BCL6	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
C1QR1	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	C1QR1	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
CCNC	FLJ32334	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	CCNC	
	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
FLJ32234	N/A	HSPCA	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
		FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	FLJ32234	
		ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
HSPCA	N/A	N/A	LAMC1	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
			HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	HSPCA	
			ABCA1	ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
LAMC1	N/A	N/A	N/A	PAIP2	IRF1	NCOA1	CLIC4	ABCA1	N/A
				LAMC1	LAMC1	LAMC1	LAMC1	LAMC1	
				ABCA1	ABCA1	ABCA1	ABCA1	ABCG1	
PAIP2	N/A	N/A	N/A	N/A	IRF1	NCOA1	CLIC4	ABCA1	N/A
					PAIP2	PAIP2	PAIP2	PAIP2	
					ABCA1	ABCA1	ABCA1	ABCG1	
IRF1	N/A	N/A	N/A	N/A	N/A	NCOA1	CLIC4	ABCA1	N/A
						IRF1	IRF1	IRF1	
						ABCA1	ABCA1	ABCG1	
NCOA1	N/A	N/A	N/A	N/A	N/A	N/A	CLIC4	ABCA1	N/A
							NCOA1	NCOA1	
							ABCA1	ABCG1	
CLIC4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ABCA1	N/A
								CLIC4	
								ABCG1	
ABCA1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ABCG1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TABLE 1

Alternative Gene Symbols	HGNC_Symbol	Locus Link ID
ABCA1	ABCA1	19
ABCG1	ABCG1	9619
ACP1	ACP1	52
ADPRT	ADPRT	142
ANGPTL2	ANGPTL2	23452
B2M	B2M	567
BCL6	BCL6	604
BMPR2	BMPR2	659
C19orf13	C19orf13	26065
C1QR1	C1QR1	22918
CCNC	CCNC	892
CLECSF6	CLECSF6	50856
CLIC4	CLIC4	25932
CLN3	CLN3	1201
DNAPTP6	DNAPTP6	26010
EBNA1BP2	EBNA1BP2	10969
EGR1	EGR1	1958
F2RL1	F2RL1	2150
FLJ11000	FLJ11000	55281
FLJ11142	FLJ11142	55779
FLJ13612	EFHD1	80303
FLJ32234	C6orf151	154007
G2AN	GANAB	23193
HSPCA	HSPCAL3	3320
HSPCB	HSPCB	3326
IKBKAP	IKBKAP	8518
IL13RA1	IL13RA1	3597

TABLE 1-continued

Alternative Gene Symbols	HGNC_Symbol	Locus Link ID
ILF1	FOXK2	3607
IRF1	IRF1	3659
LAMC1	LAMC1	3915
LCMT2	LCMT2	9836
MAFB	MAFB	9935
NCOA1	NCOA1	8648
NXN	NXN	64359
PAIP2	PAIP2	51247
PDCD5	PDCD5	9141
PDK4	PDK4	5166
PER1	PER1	5187
PF4	PF4V1	5196
PF4	PF4	5197
PMSCL2	EXOSC10	5394
PPIF	PPIF	10105
SETBP1	SETBP1	26040
SFRS6	SFRS6	6431
SLC5A6	SLC5A6	8884
TNFAIP6	TNFAIP6	7130
TSPAN2	TSPAN-2	64521
WDR9	C21orf107	54014
YES1	YES1	7525
ZFR	ZFR	51663
ZNF397	ZNF397	84307
WWP2	WWP2	11060

TABLE 2

Alternative Gene Symbols	HGNC_Symbol	RefSeq_Accession	RefSeq_Protein Accession	Locus Link ID
ABCA1	ABCA1	NM_005502	NP_005493	19
ABCG1	ABCG1	NM_207629	NP_997512	9619
ABCG1	ABCG1	NM_207628	NP_997511	9619
ABCG1	ABCG1	NM_207630	NP_997513	9619
ABCG1	ABCG1	NM_004915	NP_004906	9619
ABCG1	ABCG1	NM_207627	NP_997510	9619
ABCG1	ABCG1	NM_207174	NP_997057	9619
ABCG1	ABCG1	NM_016818	NP_058198	9619
ACP1	ACP1	NM_004300	NP_004291	52
ADPRT	ADPRT	NM_001618	NP_001609	142
ANGPTL2	ANGPTL2	NM_012098	NP_036230	23452
B2M	B2M	NM_004048	NP_004039	567
BCL6	BCL6	NM_001706	NP_001697	604
BCL6	BCL6	NM_138931	NP_620309	604
BMPR2	BMPR2	NM_001204	NP_001195	659
BMPR2	BMPR2	NM_033346	NP_203132	659
C19orf13	C19orf13	NM_015578	NP_056393	26065
C1QR1	C1QR1	NM_012072	NP_036204	22918
CCNC	CCNC	NM_005190	NP_005181	892
CLECSF6	CLECSF6	NM_194447	NP_919429	50856
CLECSF6	CLECSF6	NM_016184	NP_057268	50856
CLIC4	CLIC4	NM_013943	NP_039234	25932
CLN3	CLN3	NM_000086	NP_000077	1201
DNAPTP6	DNAPTP6	NM_015535	NP_056350	26010
EBNA1BP2	EBNA1BP2	NM_006824	NP_006815	10969
EGR1	EGR1	NM_001964	NP_001955	1958
F2RL1	F2RL1	NM_005242	NP_005233	2150
FLJ11000	FLJ11000	NM_018295	NP_060765	55281
FLJ11142	FLJ11142	NM_018338	NP_060808	55779
FLJ13612	EFHD1	NM_025202	NP_079478	80303
FLJ32234	C6orf151	NM_152551	NP_689764	154007
G2AN	GANAB	NM_014610	NP_055425	23193
G2AN	GANAB	NM_198335	NP_938149	23193
G2AN	GANAB	NM_198334	NP_938148	23193
HSPCA	HSPCAL3	XM_084514	XP_084514	3320
HSPCA	HSPCA	NM_005348	NP_005339	3320
HSPCB	HSPCB	NM_007355	NP_031381	3326
IKBKAP	IKBKAP	NM_003640	NP_003631	8518
IL13RA1	IL13RA1	NM_001560	NP_001551	3597
ILF1	FOXK2	NM_181430	NP_852095	3607
ILF1	FOXK2	NM_181431	NP_852096	3607
ILF1	FOXK2	NM_004514	NP_004505	3607
IRF1	IRF1	NM_002198	NP_002189	3659
LAMC1	LAMC1	NM_002293	NP_002284	3915
LCMT2	LCMT2	NM_014793	NP_055608	9836
MAFB	MAFB	NM_005461	NP_005452	9935
NCOA1	NCOA1	NM_003743	NP_003734	8648
NCOA1	NCOA1	NM_147233	NP_671766	8648
NCOA1	NCOA1	NM_147223	NP_671756	8648
NXN	NXN	NM_022463	NP_071908	64359
PAIP2	PAIP2	NM_016480	NP_057564	51247
PDCD5	PDCD5	NM_004708	NP_004699	9141
PDK4	PDK4	NM_002612	NP_002603	5166
PER1	PER1	NM_002616	NP_002607	5187
PF4	PF4V1	NM_002620	NP_002611	5196
PF4	PF4	NM_002619	NP_002610	5197
PMSCL2	EXOSC10	NM_002685	NP_002676	5394
PPIF	PPIF	NM_005729	NP_005720	10105
SETBP1	SETBP1	NM_015559	NP_056374	26040
SFRS6	SFRS6	NM_006275	NP_006266	6431
SLC5A6	SLC5A6	NM_021095	NP_066918	8884
TNFAIP6	TNFAIP6	NM_007115	NP_009046	7130
TSPAN2	TSPAN-2	NM_005725	NP_005716	64521
WDR9	C21orf107	NM_018963	NP_061836	54014
WDR9	C21orf107	NM_033656	NP_387505	54014
YES1	YES1	NM_005433	NP_005424	7525
ZFR	ZFR	NM_016107	NP_057191	51663
ZNF397	ZNF397	NM_032347	NP_115723	84307
WWP2	WWP2	NM_007014	NP_008945	11060
WWP2	WWP2	NM_199423	NP_955455	11060
WWP2	WWP2	NM_199424	NP_955456	11060

TABLE 2-continued

Alternative Gene Symbols	Rta_Transcript Anotation
ABCA1	*Homo sapiens* ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 7, mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 6, mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 1, mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 4, mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 5, mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 3, mRNA
ABCG1	*Homo sapiens* ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1), transcript variant 2, mRNA
ACP1	*Homo sapiens* acid phosphatase 1, soluble (ACP1), transcript variant 3, mRNA
ADPRT	*Homo sapiens* ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase) (ADPRT), mRNA
ANGPTL2	*Homo sapiens* angiopoietin-like 2 (ANGPTL2), mRNA
B2M	*Homo sapiens* beta-2-microglobulin (B2M), mRNA.
BCL6	*Homo sapiens* B-cell CLL/lymphoma 6 (zinc finger protein 51) (BCL6), transcript variant 1, mRNA
BCL6	*Homo sapiens* B-cell CLL/lymphoma 6 (zinc finger protein 51) (BCL6), transcript variant 2, mRNA
BMPR2	*Homo sapiens* bone morphogenetic protein receptor, type II (serine/threonine kinase) (BMPR2), transcript variant 1, mRNA
BMPR2	*Homo sapiens* bone morphogenetic protein receptor, type II (serine/threonine kinase) (BMPR2), transcript variant 2, mRNA
C19orf13	*Homo sapiens* chromosome 19 open reading frame 13 (C19orf13), mRNA
C1QR1	*Homo sapiens* complement component 1, q subcomponent, receptor 1 (C1QR1), mRNA
CCNC	*Homo sapiens* cyclin C (CCNC), mRNA
CLECSF6	*Homo sapiens* C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 6 (CLECSF6), transcript variant 3, mRNA
CLECSF6	*Homo sapiens* C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 6 (CLECSF6), transcript variant 1, mRNA
CLIC4	*Homo sapiens* chloride intracellular channel 4 (CLIC4), mRNA
CLN3	*Homo sapiens* ceroid-lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease) (CLN3), mRNA
DNAPTP6	*Homo sapiens* DNA polymerase-transactivated protein 6 (DNAPTP6), mRNA
EBNA1BP2	*Homo sapiens* EBNA1 binding protein 2 (EBNA1BP2), mRNA
EGR1	*Homo sapiens* early growth response 1 (EGR1), mRNA
F2RL1	*Homo sapiens* coagulation factor II (thrombin) receptor-like 1 (F2RL1), mRNA
FLJ11000	*Homo sapiens* hypothetical protein FLJ11000 (FLJ11000), mRNA
FLJ11142	*Homo sapiens* hypothetical protein FLJ11142 (FLJ11142), mRNA
FLJ13612	*Homo sapiens* EF hand domain containing 1 (EFHD1), mRNA
FLJ32234	*Homo sapiens* chromosome 6 open reading frame 151 (C6orf151), mRNA
G2AN	*Homo sapiens* glucosidase, alpha; neutral AB (GANAB), mRNA
G2AN	*Homo sapiens* glucosidase, alpha; neutral AB (GANAB), mRNA
G2AN	*Homo sapiens* glucosidase, alpha; neutral AB (GANAB), mRNA
HSPCA	*Homo sapiens* heat shock 90 kDa protein 1, alpha-like 3 (HSPCAL3), mRNA
HSPCA	*Homo sapiens* heat shock 90 kDa protein 1, alpha (HSPCA), mRNA
HSPCB	*Homo sapiens* heat shock 90 kDa protein 1, beta (HSPCB), mRNA
IKBKAP	*Homo sapiens* inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP), mRNA
IL13RA1	*Homo sapiens* interleukin 13 receptor, alpha 1 (IL13RA1), mRNA
ILF1	*Homo sapiens* forkhead box K2 (FOXK2), transcript variant 2, mRNA
ILF1	*Homo sapiens* forkhead box K2 (FOXK2), transcript variant 3, mRNA
ILF1	*Homo sapiens* forkhead box K2 (FOXK2), transcript variant 1, mRNA
IRF1	*Homo sapiens* interferon regulatory factor 1 (IRF1), mRNA
LAMC1	*Homo sapiens* laminin, gamma 1 (formerly LAMB2) (LAMC1), mRNA

TABLE 2-continued

LCMT2	*Homo sapiens* leucine carboxyl methyltransferase 2 (LCMT2), mRNA
MAFB	*Homo sapiens* v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) (MAFB), mRNA
NCOA1	*Homo sapiens* nuclear receptor coactivator 1 (NCOA1), transcript variant 1, mRNA
NCOA1	*Homo sapiens* nuclear receptor coactivator 1 (NCOA1), transcript variant 3, mRNA
NCOA1	*Homo sapiens* nuclear receptor coactivator 1 (NCOA1), transcript variant 2, mRNA
NXN	*Homo sapiens* nucleoredoxin (NXN), mRNA
PAIP2	*Homo sapiens* poly(A) binding protein interacting protein 2 (PAIP2), mRNA
PDCD5	*Homo sapiens* programmed cell death 5 (PDCD5), mRNA
PDK4	*Homo sapiens* pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4), mRNA
PER1	*Homo sapiens* period homolog 1 (*Drosophila*) (PER1), mRNA
PF4	*Homo sapiens* platelet factor 4 variant 1 (PF4V1), mRNA
PF4	*Homo sapiens* platelet factor 4 (chemokine (C—X—C motif) ligand 4) (PF4), mRNA
PMSCL2	*Homo sapiens* exosome component 10 (EXOSC10), mRNA
PPIF	*Homo sapiens* peptidylprolyl isomerase F (cyclophilin F) (PPIF), nuclear gene encoding mitochondrial protein, mRNA
SETBP1	*Homo sapiens* SET binding protein 1 (SETBP1), mRNA
SFRS6	*Homo sapiens* splicing factor, arginine/serine-rich 6 (SFRS6), mRNA
SLC5A6	*Homo sapiens* solute carrier family 5 (sodium-dependent vitamin transporter), member 6 (SLC5A6), mRNA
TNFAIP6	*Homo sapiens* tumor necrosis factor, alpha-induced protein 6 (TNFAIP6), mRNA
TSPAN2	*Homo sapiens* tetraspan 2 (TSPAN-2), mRNA
WDR9	*Homo sapiens* chromosome 21 open reading frame 107 (C21orf107), transcript variant 1, mRNA
WDR9	*Homo sapiens* chromosome 21 open reading frame 107 (C21orf107), transcript variant 2, mRNA
YES1	*Homo sapiens* v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1 (YES1), mRNA
ZFR	*Homo sapiens* zinc finger RNA binding protein (ZFR), mRNA
ZNF397	*Homo sapiens* zinc finger protein 397 (ZNF397), mRNA
WWP2	*Homo sapiens* WW domain containing E3 ubiquitin protein ligase 2 isoform 1
WWP2	*Homo sapiens* WW domain containing E3 ubiquitin protein ligase 2 isoform 3
WWP2	*Homo sapiens* WW domain containing E3 ubiquitin protein ligase 2 isoform 2

REFERENCES

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[0671] The contents of all references, patents and patent applications (including, published patent applications) cited throughout this application are hereby incorporated by reference.

Equivalents

[0672] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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gcacttctct cactgtatgt cccac 25

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aagatccaag tcgcagcagt 20

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tcccataact cctctttaac ttgtc 25

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cgagaagaga acatgaggga agga 24

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<220> FEATURE:
<223> OTHER INFORMATION: Primer - reverse primer ZFR

<400> SEQUENCE: 78

tagagcagcc agagcgtcaa 20

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<223> OTHER INFORMATION: Primer - forward primer IKBKAP

<400> SEQUENCE: 79

gcagcccagc ttaactttac ca 22

<210> SEQ ID NO 80
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<223> OTHER INFORMATION: Primer - reverse primer IKBKAP

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<400> SEQUENCE: 80

taatcctggg cacactcttc ca 22

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer - forward primer ABCA1

<400> SEQUENCE: 81

agttggcaag gttggtgagt 20

<210> SEQ ID NO 82
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer - reverse primer ABCA1

<400> SEQUENCE: 82

atggctgtag agagcttgcg tt 22

<210> SEQ ID NO 83
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<220> FEATURE:
<223> OTHER INFORMATION: Primer - forward primer ABCG1

<400> SEQUENCE: 83

tgcaggtggc cactttcgtg 20

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ccttcgaacc catacctgac a 21

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actccagcac tgtcgccat 19

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<223> OTHER INFORMATION: Primer - forward primer NCOA1

<400> SEQUENCE: 87

tccagtatcc aggagcagga a 21

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<223> OTHER INFORMATION: Primer - reverse primer NCOA1

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agaactctga ggaggaggga tt 22

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tgccctccca agtacttaaa gc 22

<210> SEQ ID NO 90
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<223> OTHER INFORMATION: Primer - reverse primer CLIC4

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cagtgcttca ttagcctctg g 21

<210> SEQ ID NO 91
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<220> FEATURE:
<223> OTHER INFORMATION: Primer - forward primer ACP1

<400> SEQUENCE: 91

tcagagaatt ggagggtaga cagc 24

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<220> FEATURE:
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<400> SEQUENCE: 92

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cccgtgacag gctacatgtt t 21

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aagggcaact tctcccaaca 20

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agacgtacaa gcaagggttt gg 22

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<223> OTHER INFORMATION: Primer - reverse primer ANGPTL2

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caccaggagt ttgtagttgc ct 22

<210> SEQ ID NO 97
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<220> FEATURE:
<223> OTHER INFORMATION: Primer - forward primer BMPR2

<400> SEQUENCE: 97

aaatagcctg gcagtgaggt 20

<210> SEQ ID NO 98
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<400> SEQUENCE: 98

ttcagccatc ctttcctcag ca 22

<210> SEQ ID NO 99
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<223> OTHER INFORMATION: Primer - forward primer C19orf13

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<400> SEQUENCE: 99

caatagagaa cggagaccaa cctg 24

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer - reverse primer C19orf13

<400> SEQUENCE: 100

acctcctctg cctctgtat 19

<210> SEQ ID NO 101
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Primer - forward primer CLECSF6

<400> SEQUENCE: 101

atatgcccgt ggaagagaca 20

<210> SEQ ID NO 102
<211> LENGTH: 22
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer - reverse primer CLECSF6

<400> SEQUENCE: 102

tgagcctcca ttctagcaca gt 22

<210> SEQ ID NO 103
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<223> OTHER INFORMATION: Primer - forward primer CLN3

<400> SEQUENCE: 103

tgccaagcat ctacctcgtc tt 22

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<400> SEQUENCE: 104

atcactggtc tccagggcga t 21

<210> SEQ ID NO 105
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Primer - forward primer DNAPTP6

<400> SEQUENCE: 105

ggtcacagaa ggcaacagac tact 24

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<210> SEQ ID NO 106
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<212> TYPE: DNA
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<400> SEQUENCE: 106

tgccttaggc ttgcttgggt ta 22

<210> SEQ ID NO 107
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<400> SEQUENCE: 107

ttccgggagt tcctgctcat tt 22

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<211> LENGTH: 22
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<223> OTHER INFORMATION: Primer - reverse primer EFHD1

<400> SEQUENCE: 108

agttcttggc acctttgaca cc 22

<210> SEQ ID NO 109
<211> LENGTH: 20
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<400> SEQUENCE: 109

gaccgcagag tcttttcctg 20

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cacaaggtgt tgccactgtt 20

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tggcaccatc caaggaacca at 22

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ttccagtgag gacagatgca ga 22

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<220> FEATURE:
<223> OTHER INFORMATION: Primer - reverse primer FLJ11000

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tgaccacaat cacgaggact 20

<210> SEQ ID NO 117
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer - forward primer FLJ11142

<400> SEQUENCE: 117

tcgtttcttg gtgactgctg ga 22

<210> SEQ ID NO 118
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer - reverse primer FLJ11142

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tccaaacctg ggagatggaa ct 22

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<223> OTHER INFORMATION: Primer - forward primer FOXK2

<400> SEQUENCE: 119

agacagcccg aaggatgatt 20

<210> SEQ ID NO 120
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<223> OTHER INFORMATION: Primer - reverse primer FOXK2

<400> SEQUENCE: 120

ttgtccgcag tcctgtagta 20

<210> SEQ ID NO 121
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Primer - forward primer HSPCB

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ccacttggca gtcaagcact tt 22

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<223> OTHER INFORMATION: Primer - forward primer LCMT2

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attcattcat gtccacggca cc 22

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cccttgtttc tttgggtgag 20

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<400> SEQUENCE: 126

acgttctcta tgcggtttgg 20

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<400> SEQUENCE: 127

tgtagattct gaggatgacg gag 23

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<223> OTHER INFORMATION: Primer - reverse primer NXN

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actcggatgc tgatgaacca 20

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aaggcatctt ggaccactgc ta 22

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taagcgtaaa tgtgcctcct cc 22

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tgacggcgga tctttcttgg t 21

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<223> OTHER INFORMATION: Primer - forward primer PF4

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aatgcacaca cgtaggcagc ta 22

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gttgctgctc ctgccactt 19

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atcgagacct ggatctgctt 20

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gacagctcct gtgacatttg gt 22

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gcagcaggtc ccagctagt 19

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tggaggtgga tgtctgttga ct 22

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atgattggcc agttcggtgt tg 22

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ttcacctgtg tctgtcctca ct 22

1.-**13**. (canceled)

14. A method of testing for mild osteoarthritis in a human test subject, the method comprising:

(a) measuring the expression level of a gene in a blood sample of the test subject, wherein the gene is tumor necrosis factor, alpha-induced protein 6 (TNFAIP6);

(b) determining whether the level of step (a) is statistically decreased compared to the expression levels of the gene in blood of human subjects not having osteoarthritis; and

(c) classifying the test subject as more likely to have mild osteoarthritis than to not have osteoarthritis if step (b) results in a determination that the level of step (a) is statistically decreased compared to the expression levels of the gene in blood of human subjects not having osteoarthritis,

thereby testing for mild osteoarthritis in a human test subject.

15. The method of claim **14**, further comprising measuring the expression levels of the gene in blood samples of control human subjects not having osteoarthritis, thereby obtaining the expression levels of the gene in blood of human subjects not having osteoarthritis.

16. The method of claim **14**, wherein measuring the expression level of the gene is done using at least one polynucleotide which can specifically and/or selectively hybridize with RNA encoded by the gene, or with DNA complementary to RNA encoded by the gene.

17. The method of claim **14**, wherein measuring the expression level of the gene is done by amplification.

18. The method of claim **14**, wherein measuring the expression level of the gene is done using the polynucleotides identified as SEQ ID NOs: 55 and 56.

19. A method of classifying a human test subject as more likely to have mild osteoarthritis than to not have osteoarthritis, the method comprising:

(a) measuring the expression level of a gene in a blood sample of the test subject, wherein the gene is tumor necrosis factor, alpha-induced protein 6 (TNFAIP6);

(b) determining whether the level of step (a) is statistically decreased compared to the expression levels of the gene in blood of human subjects not having osteoarthritis; and

(c) classifying the test subject as more likely to have mild osteoarthritis than to not have osteoarthritis if step (b) results in a determination that the level of step (a) is statistically decreased compared to the expression levels of the gene in blood of human subjects not having osteoarthritis.

20. The method of claim **19**, further comprising measuring the expression levels of the gene in blood samples of control human subjects not having osteoarthritis, thereby obtaining the expression levels of the gene in blood of human subjects not having osteoarthritis.

21. The method of claim **19**, wherein measuring the expression level of the gene is done using at least one polynucleotide which can specifically and/or selectively hybridize with RNA encoded by the gene, or with DNA complementary to RNA encoded by the gene.

22. The method of claim **19**, wherein measuring the expression level of the gene is done by amplification.

23. The method of claim **19**, wherein measuring the expression level of the gene is done using the polynucleotides identified as SEQ ID NOs: 55 and 56.

* * * * *

专利名称(译)	骨关节炎生物标志物及其用途		
公开(公告)号	US20100143916A1	公开(公告)日	2010-06-10
申请号	US12/554141	申请日	2009-09-04
申请(专利权)人(译)	GENENEWS INC.		
当前申请(专利权)人(译)	GENENEWS INC.		
[标]发明人	LIEW CHOONG CHIN YAGER THOMAS DEMPSEY ADAM CHAO SAMUEL ZHANG HONGWEI MARSHALL WAYNE		
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IPC分类号	C12Q1/68 C07K16/28 C12N C12N15/113 G01N33/53 G01N33/564		
CPC分类号	C12N15/113 C12N2310/11 C12N2310/12 C12N2310/14 G01N2800/52 C12Q1/6883 C12Q2600/158 G01N33/564 G01N2800/105 C12N2310/15		
优先权	60/493607 2003-08-08 US 60/516823 2003-11-03 US		
其他公开文献	US8142998		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及新型生物标志物的鉴定和选择以及在骨关节炎和/或骨关节炎的特定阶段中差异表达的新型生物标志物组合的鉴定和选择，以及选择新型生物标志物组合的方法。本发明的生物标志物的产物和生物标志物的组合的表达的测量在以下一个或多个中表现出特别的优势：（a）诊断个体患有关节炎，（b）区分骨关节炎（OA）的两个阶段和（c）诊断个体患有骨关节炎（OA）的特定阶段。如将理解的，为了测量本发明的生物标志物的产物，与本发明的生物标志物的产物特异性和/或选择性杂交的多核苷酸和蛋白质也包括在本发明的范围内，包含所述生物标志物的试剂盒也包括在本发明的范围内。核苷酸用于（a）诊断患有关节炎的个体，（b）区分骨关节炎（OA）的两个阶段和（c）诊断具有特定骨关节炎（OA）阶段的个体的蛋白质和蛋白质。本发明还包括多核苷酸和蛋白质的用途，其特异性地和/或选择性地与本发明的生物标记物的产物杂交，以监测个体的疾病进展并监测治疗方案的功效。本发明还提供了使用本发明生物标志物的产物鉴定骨关节炎的新治疗靶标的方法。本发明还提供了使用本发明生物标志物的产物鉴定结合和/或调节本发明基因活性的化合物的方法。通过这些方法鉴定的化合物可用于开发研究骨关节炎和骨关节炎进展的试验。此外，通过这些方法鉴定的化合物可用作开发预防和治疗组合物中的先导化合物，用于预防，治疗，控制和/或改善骨关节炎或其症状。

	Gene Name	Abbr. Gene Name	Gene Accession Numbers and UniGene Accession Numbers	Protein Accession Number	p Values or T-test Values demonstrating statistical significance
1	Beta-2-microglobulin	B2M	NM_004048; HS.48516	NP_004039	0.01
2	Alpha glucosidase II alpha subunit	G2AN	NM_014610; D42041; HS.76847	NP_055425	<0.001
3	interleukin 13 receptor alpha 1	IL13RA1	NM_001560; HS.285115	NP_001551	0.014
4	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	IKBKAP	NM_003640; BC033094; HS.31323	NP_003631	0.002
5	Tumor necrosis factor alpha-induced protein 6	TNFAIP6	NM_007115; HS.29352	NP_009046	0.003
6	WD repeat domain 9	WDR9	BC047058; NM_018963; HS.225674	Q9NSI6	0.016
7	Nedd-4-like ubiquitin-protein ligase	WWP2	NM_007014; BC013645; HS.333382	NP_008945	0.012
8	B-cell CLL/lymphoma 6	BCL6	NM_138931; HS.155024	NP_620309	0.043
9	Complement component 1, q subcomponent, receptor 1	C1QR1	NM_012072; HS.97199	NP_036204	0.048
10	Cyclin C	CCNC	NM_005190; BC050726; HS.118442	NP_005181	0.028
11	Chromosome 6 open reading frame 151	FLJ32234	NM_152551; HS.13366	NP_689764	0.041
12	Heat shock 90kDa protein 1, alpha	HSPCA	NM_005348; BC017233; HS.356531	NP_005339	0.004

FIGURE 1