

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
10 November 2005 (10.11.2005)

PCT

(10) International Publication Number
WO 2005/106495 A1

(51) International Patent Classification⁷: **G01N 33/68**,
A61K 31/00, C12Q 1/37

(21) International Application Number:
PCT/IB2005/001152

(22) International Filing Date: 21 April 2005 (21.04.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/567,979 4 May 2004 (04.05.2004) US

(71) Applicant (for all designated States except US): **PHARMACIA & UPJOHN COMPANY LLC** [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ABRAMS, Mark, Allan** [US/US]; Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017-1732 (US). **AGGARWAL, Poonam, Pereira** [US/US]; Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017-1732 (US). **DUFIELD, Dawn, Rene** [US/US]; Pfizer Global

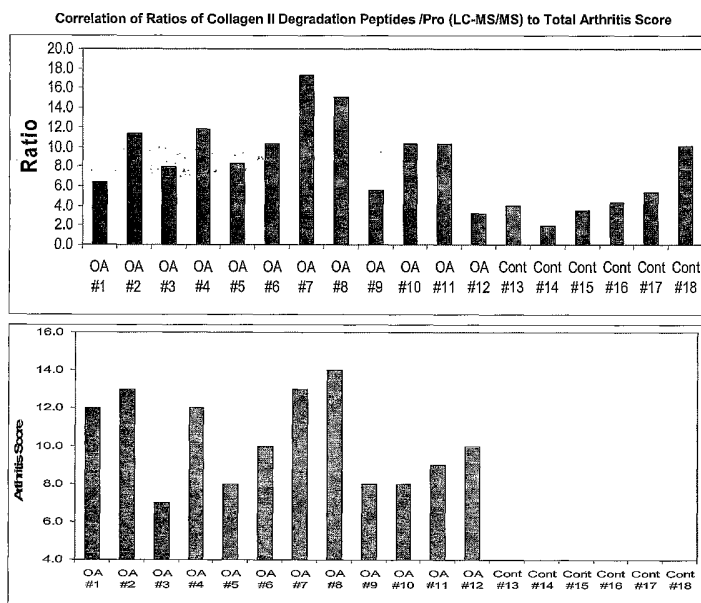
Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017-1732 (US). **MATHEWS, Rodney, W.** [US/US]; Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017-1732 (US). **NEMIROVSKIY, Olga, Vladimirovna** [US/US]; Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017-1732 (US). **SUNYER, Teresa** [US/US]; Pfizer Global Research and Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017-1732 (US).

(74) Agents: **FULLER, Grover, F., Jr.** et al.; Pfizer Inc., P.O. Box 1027, St. Louis, MO 63006 (US).

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

[Continued on next page]

(54) Title: PROCOLLAGEN II BIOMARKERS AND METHODS



(57) Abstract: A method of prediction disease progression in a disorder characterized by the uncoupling of collagen type II synthesis and degradation is disclosed. There is further disclosed a method of detecting specific procollagen fragments associated with osteoarthritis. The fragments are derived from procollagen type IIB (PIIBNP). A method for determining the efficacy of an agent or drug intended to decrease or inhibit a collagenase enzyme is disclosed.



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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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

Published:

— *with international search report*

PROCOLLAGEN II BIOMARKERS AND METHODS

SEQUENCE LISTING

5 A sequence listing is appended, including a paper copy and a copy in digital media in machine-readable form, which is identical to the paper copy.

BACKGROUND OF THE INVENTION

I. Field of the Invention

10 The present invention relates to biomarkers of arthritis, and more particularly to peptide biomarkers of collagen synthesis and degradation for diagnosing and treating osteoarthritis. The invention relates generally to methods for identifying and quantifying peptides and, more particularly, to methods for identifying and quantifying degradation peptides resulting from enzyme cleavage of collagen, and propeptide fragments resulting from collagen synthesis. The invention also relates to the specific peptides that result from synthesis of collagen type II in humans and animals and the recognition of levels
15 these peptides in biological samples as markers of diseases or physiological conditions characterized by a disequilibrium of synthesis and enzymatic degradation of collagen, such as osteoarthritis and rheumatoid arthritis, and the identification and quantification of the peptides to assess the efficacy of enzyme inhibiting agents and drugs used to treat or control such diseases or physiological conditions. The invention is further directed to quantifying degradation peptides and synthesis peptides to calculate a
20 ratio thereof, for determining disease progression and disease stage.

II. Background

Articular cartilage acts to cushion and support joints in the skeleton. Cartilage tissue features an extensive extracellular matrix. This matrix is synthesized and maintained by a single cell type, the
25 chondrocyte. Major matrix components of cartilage include collagens, particularly type II collagen, and proteoglycans, particularly the large aggregating proteoglycan, aggrecan. Type II collagen imparts mechanical strength to cartilage, while aggrecan provides compressibility and elasticity. Functionally, the fibrous collagen network enmeshes aggrecan. The negative charge conferred upon cartilage by aggrecan through its glycosaminoglycan side chains acts to retain water, while the collagen fiber network resists
30 swelling of the tissue.

When collagen type II is synthesized in adult humans, the immature protein (SEQ ID NO. 1) contains three extra domains: a signal sequence (SEQ ID NO. 2), and two propeptides, an NH₂-terminal propeptide (SEQ ID NO. 3) and a carboxy-terminal propeptide (SEQ ID NO. 4). The signal sequence directs the protein to be secreted from the cell, and the propeptides are then cleaved by specific
35 proteases. The resultant mature collagen II protein (SEQ ID NO.5) is then capable of being incorporated into the extracellular matrix.

Arthritis, particularly osteoarthritis, involves proteolytic cleavage of both collagen and aggrecan. This proteolytic cleavage is carried out by matrix metalloproteases, such as MMP-13 (a collagenase), and disintegrins, such as ADAMTS-4 / ADAMTS-5 (aggrecanases). Both MMP-13 and aggrecanases are
40 known to be upregulated in osteoarthritis.

Generally, active and extensive collagen turnover is not considered a prominent feature in healthy adults. Turnover of collagen type II, also known as articular collagen, generally occurs in diseases, such as rheumatoid arthritis, osteoarthritis or other diseases or physiological conditions in which collagen degradation is a factor.

5 The breakdown of collagen type II is believed to be initiated by specific members of the matrix metalloproteinase family of enzymes, the collagenases. Collagens are comprised of three peptide strands wound in a helix formation. For example, collagen type II is comprised of a triple helix of three identical peptide strands referred to as peptide alpha 1, collagen type II. When collagen is degraded or
10 cleaved by a collagenase, the cleavage takes place at a specific intra-helical site leaving the peptides vulnerable to further degradation or cleavage. In any event, the initial cleavage results in the generation of collagen fragments having an end defined by the proteolytic cleavage. For example, collagenase degradation of collagen type II results in a peptide fragment having the C-terminal sequence ending with: GPXGPQG, where X is proline or hydroxyproline. Billingham et al described this primary collagenase
15 cleavage site and developed antibodies reactive to both the carboxy-terminal and amino-terminal "neoepitopes" generated by cleavage of native human collagen type II. (*J. Clin. Invest.* 99:1534-1545 (1997)). Otterness et al have described production of a monoclonal antibody directed against this carboxy-terminal "neoepitope" (*Matrix Biology* 18: 331-341 (1999)).

U.S. Patent No. 6,030,792 to Otterness et. al. discloses antibodies for detecting collagen fragments resulting from collagenase cleavage of type II collagen. Poole et al. U.S. Patent No. 6,132,976
20 provides a method for evaluating cartilage degradation by immunoassay measurement of type II collagen cleavage. These patents and references describe methods for the detection of collagen type II enzymatic cleavage by detecting the presence of peptide fragments only determine the presence of the target C-terminus portion and immediately adjacent peptide sequence using antibodies specific for these sequence regions. WO 03/021226, the disclosure of which is incorporated herein by reference, describes
25 collagen type II fragment biomarkers of diseases from proteolytic cleavage of collagen type II by metalloproteinase enzymes and methods of identifying and quantifying such peptide fragments.

Recently, it has been discovered that in an early stage of osteoarthritis, chondrocytes proliferate, and synthesize more collagen, presumably to compensate for the loss of collagen type II due its degradation by collagenases. A specific collagen, type IIA, was first identified in developing embryos
30 from chondroprogenitor cells in developing skeletal tissues. Type IIA procollagen (SEQ ID NO. 6) is characterized by a cysteine-rich region in the NH₂ terminal end, termed PIIANP. Normal adult chondrocytes, in contrast, do not express NH₂-terminal type IIA procollagen, but rather express NH₂-terminal type IIB procollagen (SEQ ID NO. 3), which lacks the NH₂ cysteine-rich sequence present in PIIANP. Aigner et al. describe a reexpression of type IIA procollagen in adults suffering from
35 osteoarthritis (*Arthritis & Rheumatism* (1999) Vol 42, No.7, pp. 1443-1450), and Sandell, U.S. Patent No. 5,780,240 describes detection of type IIA procollagen mRNA or peptide associated with osteoarthritis in adult humans.

Garnero et al. have proposed that a disequilibrium between synthesis of new collagen, characterized by embryonic pro-collagen IIA production and degradation of articular collagen is predictive of osteoarthritis severity and progression. *Arthritis & Rheumatism* (2002) Vol. 46, No. 10, pp. 2613-2624. In that study,
40 type IIA procollagen (PIIANP) levels were detected in serum using an ELISA, and C-terminal cross linked

telo peptide (CTX-II) were detected in urine using an ELISA, at a baseline and one year time points. A ratio of PIIANP and CTX-II was calculated, and the result was termed an "uncoupling index." Patients diagnosed with osteoarthritis and having relatively low detected levels of PIIANP and high levels of CTX-II exhibited a greater level of disease progression over one year, as determined by radiography, than those patients with either high levels of PIIANP or low levels of CTX-II, although increased levels of CTX-II was determined to be a better predictor of disease progression than relatively high levels of PIIANP. Serum levels of PIIANP, however, are not necessarily correlated to PIIANP levels in synovial fluid. Furthermore, PIIANP has not been identified in urine. Therefore, there is a need for an assay that measures peptides from a consistent biological fluid or tissue that provides correlation between peptide levels and disease progression.

SUMMARY OF THE INVENTION

There is now disclosed a method of predicting disease progression in a disorder characterized by the uncoupling of collagen type II synthesis and degradation. There is further disclosed a method of detecting specific procollagen fragments associated with osteoarthritis. The present inventors were able to detect PIIBNP fragments in the urine of both normal and osteoarthritis patient urine samples.

Accordingly, an embodiment of the present invention is directed to a method of determining the level of type II B procollagen fragments (PIIBNP, synthesis products) in a biological sample, determining the level of type II collagen degradation fragments (degradation products) in a biological sample, and calculating a ratio of synthesis products to degradation products to determine disease state or predict disease progression. In an embodiment of the present invention, PIIBNP synthesis products and type II collagen degradation products are both collected from urine. The selection of urine as a biological sample provides a less invasive, easier to obtain source of biomarkers than previously described methods. It is believed that obtaining biomarkers from a consistent source will result in improved diagnosis, prognosis, quantification and characterization, since these collagen fragments may be subject to further proteolytic degradation in different portions of the body after their initial cleavage *in vivo*. In another embodiment of the present invention, specific PIIBNP fragments are characterized and differentiated between healthy adults and adults afflicted with an arthritis condition.

In another embodiment of the present invention, the level of type II procollagen fragments (PIINP, synthesis products) is determined in a biological sample, the level of type II collagen degradation fragments (degradation products) is determined in a biological sample, and a ratio of synthesis products to degradation products is calculated to determine disease state or predict disease progression. In an embodiment of the present invention, PIINP synthesis products and type II collagen degradation products are both collected from urine. The selection of urine as a biological sample provides a less invasive, easier to obtain source of biomarkers than previously described methods. It is believed that obtaining biomarkers from a consistent source will result in improved diagnosis, prognosis, quantification and characterization, since these collagen fragments may be subject to further proteolytic degradation in different portions of the body after their initial cleavage *in vivo*.

In another embodiment of the present invention, specific peptides detected in urine are disclosed. These peptides are QDVRQPGPKGQKGEPGD (SEQ ID NO. 7); QDVRQPGPKGQKGEPGD (SEQ ID NO. 8); QDVRQPGPKGQKGEPGDIKDIK (SEQ ID NO. 9); QDVRQPGPKGQKGEPGDIKDIVGPKGPPGP

(SEQ ID NO. 10); QDVRQPGPKGQKGEPGDIKDIVGPKGPPGPQGPGAG (SEQ ID NO. 11); and QDVRQPGPKGQKGEPGDIKDIVGPKGPPGPQGPGPAGEQGPRG (SEQ ID NO. 12), including post translational modifications and derivatives thereof.

The present invention is further directed to a method of determining the efficacy of an agent or drug administered to treat a disease or condition mediated by pathological degradation of type II collagen, particularly osteoarthritis. Another embodiment of the present invention relates to a method of determining the efficacy of an agent or drug intended to decrease or inhibit a collagenase enzyme comprising:

obtaining a first biological sample from a subject with a collagen type II degradation mediated disorder;

detecting a PIIBNP in the first biological sample;

quantifying the concentration of the PIIBNP in the first biological sample,

detecting a collagen type II degradation peptide in the first biological sample;

quantifying the concentration of the collagen type II degradation peptide;

administering an agent or drug intended to decrease or inhibit a collagenase enzyme;

obtaining a second biological sample from the subject;

quantifying the concentration of the PIIBNP in the second biological sample,

detecting a collagen type II degradation peptide in the second biological sample;

quantifying the concentration of the collagen type II degradation peptide; and

comparing the concentration of the PIIBNP and the concentration of the collagen type II degradation peptide from the second sample with the concentration of the PIIBNP and the concentration of the collagen type II degradation peptide from the first sample to determine the efficacy of the agent or drug intended to decrease or inhibit a collagenase.

Yet another embodiment of the present invention relates to a method of determining an appropriate dose of an agent or drug intended to decrease or inhibit a collagenase enzyme. To this end, the present invention may also be useful in determining an appropriate dose of an agent or drug, tailored to an individual's relative disease state and to an individual's sensitivity to such agent or drug. This embodiment is directed to a method comprising:

obtaining a first biological sample from a subject with a collagen type II degradation mediated disorder;

detecting a PIINP in the first biological sample;

quantifying the concentration of the PIINP in the first biological sample,

detecting a collagen type II degradation peptide in the first biological sample;

quantifying the concentration of the collagen type II degradation peptide;

administering an agent or drug intended to decrease or inhibit a collagenase enzyme in a fixed dose;

obtaining a second biological sample from the subject;

quantifying the concentration of the PIINP in the second biological sample,

detecting a collagen type II degradation peptide in the second biological sample;

quantifying the concentration of the collagen type II degradation peptide; and

comparing the concentration of the PIINP and the concentration of the collagen type II degradation peptide from the second sample with the concentration of the PIIBNP and the concentration of the collagen type II degradation peptide from the first sample to determine the appropriate dose of the agent or drug intended to decrease or inhibit a collagenase.

5 In addition, prior art biomarkers and methods may be used in combination with the PIINP biomarkers and methods of the present invention.

 Further advantages of the present invention will be apparent to those of skill in the art, in light of the disclosure herein.

BRIEF DESCRIPTION OF THE DRAWINGS

5 FIG. 1 shows the performance of a ZCGPKGQKGEPGDIKD epitope generated antibody column.

FIG 2 shows a generalized process for detecting procollagen peptides obtained from a biological sample;

10 FIG 3 is a mass spectrum of a peptide fragment, QDVRQPGPKGQKGEPGDIKD (SEQ ID NO. 9) obtained from the urine of a child;

FIG 4 is a mass spectrum of a peptide fragment QDVRQPGPXGQKGEPGDIKD, with three hexose sugar post-translational modification, where X is hydroxyl-allysine;

15 FIG 5 is a graphic representation comparing the concentration of collagen peptide fragments obtained from twelve osteoarthritis patients to collagen peptide fragments obtained from age-matched healthy control patients;

20 FIG 6 is a graphic representation comparing the ratio of collagen type II degradation peptides to procollagen NH₂-terminal synthesis peptides (PIIBNP) between osteoarthritis patients and age-matched healthy control patients; and

25 FIG 7 is a graphic representation of a correlation between the ratio of collagen type II degradation peptides to procollagen NH₂-terminal synthesis peptides (PIIBNP) and arthritis score from osteoarthritis patients; and

FIG 8 is a graphic representation of a competition assay of an embodiment of the present invention.

30

DETAILED DESCRIPTION OF THE INVENTION

The following detailed description is provided to aid those skilled in the art in practicing the present invention. Even so, this detailed description should not be construed to unduly limit the present invention as modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present inventive discovery.

The contents of each of the references cited herein, including the contents of the references cited within these primary references, are herein incorporated by reference.

a. Definitions

The following definitions may be applied to terms employed in the description of embodiments of the invention. The following definitions supercede any contradictory definitions contained in each individual reference incorporated herein by reference.

Nucleic acids, amino acids, peptides, protective groups, active groups and similar moieties, when abbreviated are abbreviated according to the IUPACIUB (Commission on Biological Nomenclature) or the practice in the fields concerned.

The following are examples.

General Abbreviations

HPLC	High pressure liquid chromatography
LC	Liquid chromatography
MS	Mass Spectrometry or Mass spectroscopy
MS/MS	Tandem mass spectrometry
MALDI	Matrix assisted laser desorption ionization
TOF	Time-of-flight
FAB	Fast atom bombardment
CF-FAB	Continuous flow fast atom bombardment
M/Z	Mass-to-charge ratio
Da	Dalton, a unit of molecular mass equal to $1/12$ mass of a ^{12}C atom.
mw	Molecular weight
mg	milligrams
mL	milliliters
pg	picograms
M	moles, molar
nM	nanomoles, nanomolar
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
Oligo	Oligonucleotide
RT	Room temperature, about 22°C

CAb Capture antibody
 DAb Detection antibody

Reagents

EDTA Ethylenediamine tetraacetic acid
 5 SDS Sodium dodecylsulfate
 TW-20 Tween-20
 NFDM Non-fat dry milk
 DPBS Dulbecco's phosphate buffered saline
 Bt Biotinylated
 10 HAT Hypoxanthine, aminopterin, thymidine containing media
 HT Hypoxanthine, thymidine containing media
 HRP Horseradish peroxidase

Immunoglobulin-like molecules or chains

15 V_H or VH: Variable region of the heavy chain
 V_L or VL: Variable region of the light chain
 scFv A single chain antibody containing a V_L region and a V_H region

20

Nucleic Acids

RNA Ribonucleic acid
 DNA Deoxyribonucleic acid
 cDNA Complimentary DNA
 25 mRNA Messenger RNA

Nucleic acid bases

	<u>Purines</u>	<u>Pyrimidines</u>
30	A: Adenine	T: Thymine
	G: Guanine	C: Cytosine
		U: Uracil

Amino Acids-Single letter codes: Three letter codes: Full names

	G:	Gly:	glycine
	V:	Val:	valine
35	L:	Leu:	leucine
	A:	Ala:	alanine
	I:	Ile:	isoleucine
	S:	Ser:	serine
	D:	Asp:	aspartic acid
40	K:	Lys:	lysine
		Hyl	hydroxylysine

	aK		allysine
		aHyl	hydroxyallysine
	R:	Arg:	arginine
	H:	His:	histidine
5	F:	Phe:	phenylalanine
	Y:	Tyr:	tyrosine
	T:	Thr:	threonine
	C:	Cys:	cysteine
	M:	Met:	methionine
10	E:	Glu:	glutamic acid
	W:	Trp:	tryptophan
	P:	Pro:	proline
	O:	Hyp:	hydroxyproline
		4Hyp	4-hydroxyproline
15	N:	Asn:	asparagine
	Q:	Gln:	glutamine
	X:	Xaa	unspecified amino acid
	Z:	---	linking group

20 **Definitions**

The term "identification," as used herein, means the characterization of a material, such as a peptide. The characterization may differentiate the material from other materials in a matrix, such as a liquid sample.

25 The term "quantification," as used herein, means a determination of relative concentration or amount of a material, such as a peptide, found in a matrix, such as a liquid sample.

The terms "detection" and "detecting," as used herein, means identification, quantification, or both.

30 The term "matrix," as applied to a biological system, means a surrounding substance within which a material, such as a peptide, originates, develops, or is contained. The surrounding substance may be a liquid or a tissue, for example.

The term "Liquid Chromatography - Mass Spectrometry (LC/MS)," as used herein, means the process of separation of complex mixtures of non-volatile compounds before introduction into a mass spectrometer. LC/MS is used for compounds that have a high molecular weight or are too heat sensitive to be analyzed by gas chromatography (GC). The most common ionization methods that are interfaced to LC are electrospray ionization (ESI) and Atmospheric Chemical Ionization (APCI) in positive and negative-ion modes. The LC is performed in most cases by RP-HPLC, and the buffer system should not contain involatile salts (such as phosphates). ESI can be used for m/z 50-4000 and is done at low resolving power.

40 The terms "Tandem MS" or "MS/MS," as used herein, refers to a process in which the ion of interest is selected with a first analyzer (MS-1), collided with inert gas atoms in a collision cell, and the fragments generated by the collision are separated by a second analyzer (MS-2). Tandem mass

spectrometry is used for structure determination of the peptides of interest. In Ion Trap and Fourier transform experiments, the experiments are carried out in one analyzer, and the various events are separated in time, not in space. The information is used to sequence peptides.

5 In order to increase sample analysis throughput, the use of fast liquid is used for structure determination of molecular ions or fragments. In Tandem MS, chromatography in quantitative bioanalysis based on liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) has become prevalent.

The term PIIBNP means procollagen type IIB NH₂-terminal peptide or peptides, such that the term covers both the singular and plural.

10 The term PIIANP means procollagen type IIA NH₂-terminal peptide or peptides, such that the term covers both the singular and plural.

The term PIINP means either PIIBNP or PIIANP or both.

15 The term "MEROPS," as used herein, means a system of classification of peptidases, and a database of peptidases available on the world wide web at address: <http://merops.sanger.ac.uk/>, and used in the text Handbook of Proteolytic Enzymes, Barrett, Alan and Rawlings, Neil, ed., Academic Press 1998 (ISBN 0-12-079370-9).

20 The term "MMP," or "matrix metalloproteinase," as used herein, means an enzyme designated clan MB (metzincins), family M10, subfamily A, by the Enzyme Commission, Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), and is a member of the family of zinc coordinating endopeptidases that function to degrade extracellular matrix proteins. MMPs are also known as matrixins.

The term "MMP-13," as used herein, means the MMP classified as clan MB, family M10, MEROPS ID M10.013. MMP-13 is also known as collagenase-3.

25 The term "MMP inhibitor," as used herein, means an agent capable of reducing or eliminating the catalytic activity of a matrix metalloproteinase, without regard to specific MMP inhibition. Thus, an MMP inhibitor may reduce or eliminate the catalytic activity of any member of the matrix metalloproteinase family, or all members of the matrix metalloproteinase family.

30 The term "MMP-13 inhibitor," as used herein, means an MMP inhibitor that is capable of reducing or eliminating the catalytic activity of MMP-13, without regard to specificity with respect to other members of the matrix metalloproteinase family. Thus, an MMP-13 inhibitor may selectively inhibit substantially only MMP-13, but spare other members of the matrix metalloproteinase family, or may alternatively inhibit any number of matrix metalloproteinase enzymes, as long as MMP-13 is inhibited. Those skilled in the art will recognize that with many selective enzyme inhibitors, the particular enzyme inhibiting agent is considered selective at a given dose range, and levels of the enzyme inhibiting agent exceeding that dose range may result in loss of selectivity.

35 The term "agent or drug intended to decrease or inhibit a collagenase" means a molecule or collection of molecules that reduces or eliminates the expression, translation or transcription of at least one collagenase, as well as a molecule or collection of molecules that directly inhibits at least one collagenase enzyme.

40 The term "immunoglobulin," or "Ig," as used herein, means a natural or genetically engineered tetrameric protein composed of two light chains of about 23 kD and two heavy chains of about 53-70 kD,

depending on the amino acid sequence and degree of glycosylation. Multimers of the tetrameric protein are also formed (IgM and IgA). There are two classes of light chains, kappa (κ) and lambda (λ), and several classes of heavy chains gamma (γ), mu (μ), alpha (α), delta (δ) and epsilon (ϵ). There are also subclasses. Each chain, whether a light chain or heavy chain, is made up of two parts. The first part, beginning from the N-terminus of either chain, is called the variable domain. The C-terminal half of the light chain is called the constant region of the light chain and it is the primary determinant whether the light chain is a κ or λ type. The constant region of the heavy chain comprise about the C-terminal three-fourths of the heavy chain and determines the class of the immunoglobulin molecule (such as, for example, IgG₁ or IgM), that is to say, a γ heavy chain corresponds to an IgG and a μ heavy chain corresponds to an IgM, for example.

V_L and V_H : The amino acid sequence of the variable domain of the light chain (V_L) and the variable domain of the heavy chain (V_H) together determine the binding specificity and the binding (kD) constant of the immunoglobulin molecule. The variable domain comprises about half the length of the light chain and about a quarter of the length of the heavy chain and for both chains, begins at the N-terminus of the chain. The variable regions each contain three (3) hypervariable segments known as the complementarity determining regions or CDRs.

CDR and FR: Each variable domain, V_L or V_H , is comprised of three CDRs: CDR1, CDR2 and CDR3. The intervening sequence segments before, between and after the CDRs are known as framework segments (FR). Each V_L and V_H is comprised of four FR segments FR1, FR2, FR3 and FR4.

V_K and V_λ : The V_L domain is either κ or λ , depending on which constant region (C_K or C_λ) is used during the productive rearrangement of the light chain (VJC_K or VJC_λ).

The term "antibodies," as used herein, means specific immunoglobulin molecules produced by B-cells of the immune system in response to challenges by proteins, glycoproteins, virus cells, chemicals coupled to carriers, and other substances. An antibody is simply an immunoglobulin molecule for which its binding partner is known. The substance to which the antibody binds is called an antigen. The binding of such antibodies to its antigen is highly refined and there are a multitude of specificities capable of being generated by changes in amino acid sequence in the variable domains of the heavy and light chains.

The term "polyclonal antibodies," as used herein, means that a variety of different antibodies are present which may be directed to different portions of a given antigen. Normal immunization leads to a wide variety of antibodies against the same antigen. Although each B lymphocyte normally produces one immunoglobulin molecule of a defined amino acid sequence, in an immune response, many B lymphocytes are stimulated to make immunoglobulin molecules that react with the antigen, that is to say, antibodies. These different antibodies are characterized by different amino acid sequences in the variable regions of the immunoglobulin molecule which result in differences in the fine specificity and affinity of binding. Such antibodies are called polyclonal antibodies to emphasize the variety of binding specificities and binding constants which arise from the variety of amino acid sequences found in the different immunoglobulin molecules utilized in the immune response.

The term "monoclonal antibodies," or "Mab," as used herein, means a plurality of antibodies that are specific to the same portion of an antigen. A B lymphocyte producing a single antibody molecule can be hybridized with an immortal B lymphocyte cell line, such as a myeloma, to derive an antibody producing immortal cell line, that is to say, a hybridoma. The hybrids thus formed are segregated into

single genetic strains by selection, dilution, subcloning, and regrowth, and each strain thus represents a single genetic line. It produces a single antibody of a unique sequence. The antibody produced by such a cell line is called "monoclonal antibody" or MAb, referencing its pure genetic parentage and differentiating it from polyclonal antibody, produced from a mixed genetic background, that is to say, multiple B cells.

5 Because a MAb is a pure chemical reagent it gives consistent, uniform results in immune tests. Moreover, because the MAb is produced by an immortal cell line, reagent supply is not limiting. For these reasons, a MAb is generally preferred over polyclonal antibodies for diagnostic purposes.

The term "genetically engineered antibody," as used herein, means an artificially created antibody with variable regions similar to those of the naturally occurring antibody, but with altered constant regions.

10 As the binding specificity of an antibody resides in the variable regions of the light and heavy chains, antibodies can be genetically engineered to change or remove the constant regions and, if done properly, it can result in an antibody molecule with different properties and molecular weight, but with the same or very similar antigen binding properties. For example, the V_L and V_H (or V_H and V_L) genes can be cloned and assembled with an appropriate linker between them. Such a new genetically engineered molecule is
15 called a single chain antibody (abbreviated scFv) and typically has a molecular weight of about 25 to 28 kD, depending on the design of the linker and the addition of other sequences to help in purification, stability, trafficking, detection, and the like. Multimers of the single chain antibody can also be made by appropriate use of linkers in which the order of each V_H and V_L pair may vary. In addition, some changes in the amino acid sequence of the V_L and V_H region can be made that retain desirable antigen binding
20 properties. It can be seen that an almost infinite variety of genetically engineered antibodies can be derived from the original antibody sequence which retain binding specificity to the antigen, but which are tailored to fulfill specific characteristics. Other examples of genetically engineered antibodies include, but are not limited to: Fab, $F(ab')_2$, chimeric antibodies, humanized antibodies, and the like.

The term "bispecific antibody, as used herein, means a discreet antibody with two binding sites of
25 different specificity. Normally an IgG antibody has two identical light chains and heavy chains. There are therefore two identical antibody binding sites in the immunoglobulin molecule. By contrast, a bispecific antibody is a single immunoglobulin molecule which has two specificities. It can be made by fusion of two monoclonal antibody producing hybridoma cell lines, where each hybridoma has a different antigen specificity, and selection for a cell line (a quadroma) that produces an antibody whose composition is a
30 tetramer composed of one light chain and one heavy chain from each hybridoma fusion partner. The antibody produced by the quadroma has only one light and one heavy chain of each parental specificity and has one binding site for each heavy/light chain pair and is bispecific, i.e., it has two binding sites of different specificities. A bispecific antibody can also be made by genetic engineering. It can comprise the V_L linker V_H of one antibody linked through an additional linker to a V_L linker V_H of another antibody
35 molecule. The order of V_L and V_H can be altered, but the end result is a bispecific antibody.

The term "epitope," as used herein, means a portion of an antigen to which an antibody specifically binds. Depending on the size, structure and conformation of the antigen, an antibody may bind only to a small part of the entire structure. The part of the antigen molecule to which the antibody binds is called its epitope. Different antibodies may be mapped to different epitopes on the same antigen.

40 The term "neoepitope," as used herein, means an epitope that is revealed after processing of a protein or peptide. The antigen may have an epitope which is hidden so that it cannot bind to a specific

antibody. However, a conformational change in the antigen may cause the appearance of the epitope by unfolding or uncovering part of the surface of the molecule. This now allows the antibody to bind to the epitope. In another aspect, the action of an enzyme on the antigen may cause the appearance of a new epitope to which the antibody can bind. For example, after cleavage by a proteolytic enzyme, new N-terminal and new C-terminal sequences are generated. Because the epitope is not observed in the parent molecule and because, after some change in the parent molecule, the epitope is revealed and now can bind antibody, it is called a neoepitope. While the term neoepitope is described in terms of antibody recognition, as used herein the term neoepitope means any such fragment of a protein or peptide, whether or not the fragment is actually determined through immunological or other means, such as, for example, mass spectroscopy. Such a peptide fragment is not found as the native sequence, but is rather formed by enzymatic cleavage of a larger protein, thus forming a new N-terminus, a new C-terminus, or both.

The term "biological media," as used herein, means any biological sample, such as a fluid, that might contain the peptide and be of interest to assay by the methods of the present invention. Biological media include, without limitation: blood, synovial fluid, urine, spinal fluid, bronchiolar lavage fluid, lymph, the vitreous humor of the eye, extracts of tissues, tissue culture supernatants, extracts of cartilage, and the like. Biological media need not be limited to human samples, but may also be obtained from a similar variety of animal media in a fashion similar to the examples above.

The term "immunoassay," as used herein, means an assay for a substance, such as a complex biological such as a protein, or a small molecule, based on employing the binding properties of an antibody to recognize the substance, which may be a specific molecule or set of homologous molecules. The immunoassay may involve one or more antibodies. Non-limiting examples of applicable immunoassays are provided below.

It is noted that the post-translational modifications, 4-hydroxylation of proline and hydroxylation of lysine, appear in varying levels in collagen peptides and peptide fragments in different species. In addition, the conversion of lysine to allysine by lysyl oxidase, and the conversion of hydroxylysine to hydroxyallysine was found in some of the procollagen peptide fragments. The originally translated peptides themselves, as well as permutations of the post-translational modifications, may be found in urine of subjects and are included among the embodiments of the invention. Identifiable derivatives or modifications of the peptides also can function as markers and are included within the scope of the invention.

Direct Assay

In a "direct assay," the antibody binds directly to an antigen such as in a biological specimen (cells, tissues, histological section, and the like) or to antigen adsorbed or chemically coupled to a solid surface. The antibody itself is usually labeled to enable the determination of the amount of antibody bound to the antigen. Alternatively, the antibody (now termed primary antibody) is detected with a secondary labeled antibody that will demonstrate that binding of the primary antibody had occurred.

Competitive Assay

An assay based on the binding properties of a single antibody molecule. Typically, a labeled

antigen is used to compete with an unknown antigen and the amount of unknown antigen is determined in terms of how much of the labeled antigen is displaced by the unknown antigen. The label may be radioactive, optical, enzymatic, fluorescent polarizing, fluorescent quenching, or other label. The antibody may be monospecific or bispecific.

5

Sandwich Assay

This is a double antibody assay in which both antibodies bind to the antigen, forming a trimeric immune complex or sandwich containing the two antibodies with the antigen between them. One antibody is utilized to localize the immune complex to the detection surface or chamber. This antibody is termed the capture antibody. The other antibody bears a label that will allow the immune complex to be detected. It is called the detection antibody. If an immune complex is not formed (no antigen is present), then the capture antibody is unable to bring the detection antibody to the detector. If antigen is present, then an immune complex will form and the capture antibody will be joined with the detection antibody such that the amount of detection antibody in the immune complex is quantitatively related to the amount of antigen present.

The assay can be formatted in many ways. For example, the capture antibody can be chemically coupled to a solid surface, or non-specifically adsorbed to a surface, attached via biotinylation to an avidin-like molecule, for example, without limitation, avidin, streptavidin, neutravidin, and the like, streptavidin or avidin-coated surface, coupled to magnetic particles or beads as a means of localizing the immune complex to the measurement device.

The detection antibody may be radiolabeled, or it may have a variety of possible enzymatic amplification systems such as horseradish peroxidase (HRP), alkaline phosphatase (AP), urease, and the like, when formatted as an Elisa (Enzyme-linked immune assay). It may have an electrochemical, an optical, a fluorescent or other detection method to determine the amount of detection antibody in the immune complexes.

It may immediately be seen that many examples can be derived in which the two antibodies are paired in a sandwich assay using a variety of methods to capture the immune complex in a detection device and a variety of detection systems to measure the amount of immune complex.

Molecular Biology Techniques

A skilled artisan could *in vitro* produce a complete gene coding for the V_H and V_L regions and a completely functional antibody. It can be produced as an immunoglobulin molecule of any given class with constant regions of the heavy chain and light chain added or it can be produced as a scFv with V_H and V_L joined by a linker with tags added as appropriate. The constructed gene may be engineered by conventional recombinant techniques, for example, to provide a gene insert in a plasmid capable of expression. Thereafter, the plasmids may be expressed in host cells where the host cells may be bacteria such as *E. coli* or a *Bacillus* species, yeast cells such as *Pichia pastoris* or in mammalian cell lines such as, for example and without limitation, Sp2/0, Ag8 or CHO cells.

40

Preferred Embodiments

One embodiment of the invention is directed to the identification and quantification of collagen peptides, particularly in biological samples from humans or animals. The term "biological sample" includes a sample from any body fluid or tissue. The invention includes a method of determining the presence of, and identifying the structure of, cleaved propeptide collagen type IIB fragments, and peptide degradation products of specific collagenase enzyme activity.

The present invention can be used to detect cleavage by proteolytic enzymes, such as, for example, matrix metalloproteinases -1, -8, and -13, that results in peptides with C-terminal amino acid sequences having characteristic fragment ions upon collisional activation by tandem mass spectrometry that can be identified by the methods of the present invention. The present invention allows the diagnosis and prognosis of physiological conditions characterized by collagen degradation through the identification and quantification of propeptide collagen type II fragments and collagen degradation peptides discovered to be present in biological samples of subjects exhibiting signs and symptoms of osteoarthritis. This quantification of the peptides can be used for diagnosis or prognosis of diseases such as osteoarthritis, to monitor collagenase enzyme activity in disease or physiological conditions characterized by collagenase activity, and to evaluate drugs or agents used to inhibit collagen degradation, such as matrix metalloproteinase inhibitors, or the levels of active collagenase, particularly those drugs or agents that result in the inhibition of collagen degradation. The identification and quantification of the biomarker peptides can be used in diagnosis and prognosis of diseases or conditions characterized by the ratio of collagen type II propeptide (as a surrogate for collagen synthesis) and collagen type II fragments (as a surrogate for collagen type II degradation).

Purification of Samples

It may be desirable to purify a biological sample by selective removal of target procollagen peptide fragments from the biological sample prior to detection. This is useful to remove other peptide fragments present in the biological sample, which would otherwise create "background noise" in mass spectrometry.

The peptides can be separated from the biological matrix components of the sample by an appropriate separation method known to the art. For example, chromatographic or electrophoretic separation can be used in this step. Other appropriate separation or "clean up" methods are contemplated by the invention. In liquid chromatographic separation the eluant containing the peptides of the target mass are introduced to a mass spectrometer through an appropriate liquid chromatography/mass spectrometry interface.

Purification may be accomplished, for example, by application of a liquid chromatography column containing antibodies specific to a known region of the propeptide of interest.

Example I – Purification of a Peptide Fragment in a Biological Sample

An antibody was raised in chickens to the artificial peptide KLH-ZCGPKGQKGEPGDIKDI (SEQ ID NO. 13) of the NH₂-terminal sequence of PIINP, where "Z" represents a linking group, and "KLH" represents Keyhole Limpet Hemocyanin, using well known techniques. The resulting antibodies were immobilized into columns using epoxide chemistry. Urine samples were applied to the column, and the bound PIINP were eluted into a sample vials. FIG. 1 shows the performance of the antibody column.

In a similar manner, an antibody was raised in chickens to the artificial peptide DQAAGGLRQHDAEVDATLCZ-KLH (SEQ ID NO. 14) of the carboxy-terminal propeptide of collagen type II, where "Z" represents a linking group, and "KLH" represents Keyhole Limpet Hemocyanin. While usable, this purified carboxy-terminal peptide did not provide as strong a signal as did the NH₂-terminal

Determination of Molecular Mass

Mass spectrometers measure mass-to-charge ratios (m/z) of ions produced from molecules introduced into an ion source. In many ionization methods developed for mass spectrometry, the charge is generally (but not always) unity, so it is often stated that 'mass' is measured with a mass spectrometer, although this is not strictly correct. Several newer ionization techniques routinely produce multiply-charged ions; most notable among these being electrospray ionization (ESI). In the case of peptides, a wide distribution of charges may be recorded for a peptide. Fortunately, these sequentially charged (m/z) states give peaks in the spectrum that can be mathematically processed to derive the original molecular mass of the peptide.

Example II - Detection of PIIBNP Fragments in Urine

Figure 2 shows the general format used in the detection of PIIBNP from a biological sample. It is to be understood that this is only an exemplary method of detecting PIIBNP, and other analogous techniques could be employed. For example, a single antibody column could be employed, or no purification step may be performed.

A sample of urine was injected at a rate of between one and two mL per minute using an Agilent 1100 quaternary pump with mobile phase A: 25 nM ammonium acetate, pH 7 through a series of up to five columns in which antibodies to the peptide sequence ZCGPKGQKGEPGDIKDI (SEQ ID NO. 13) of the NH₂-terminal sequence of PIIBNP was immobilized by either Protein G or epoxide coupling to the resin therein, into an autosampler with a 96 well plate format into 1ml vials. An elution with mobile phase B: 0.1 – 0.5% formic acid or ammonium acetate at pH 4.5 was performed on the column, and the resulting samples were eluted with 300 μ L/min. flow rate onto a Betasil C-18 column (ThermoHypersil-Keystone Co., Bellefonte, PA, 5 micron particle size, 100 x 2 mm) with mobile phase composed of with 1% formic acid using a second Agilent 1100 quaternary pump, and collected in a Michrom Bioresources macro peptrap, a C-18 type trap, (Michrom BioResources, Inc., Auburn, CA) that was connected to an HP1100 HPLC system (Agilent, Palo Alto, CA). Waste was expelled by a wash with mobile phase B: 1% formic acid in acetonitrile. The purified samples were analyzed in an API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA 94404, U.S.A.) operated in MRM mode with detection limits between 100 pg/mL – 100 ng/mL.

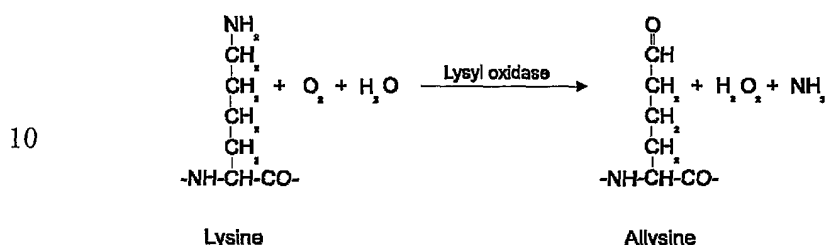
The peptides are fragmented by collisional activation by a neutral gas in a collision cell using collision energies and methods known to the art resulting in collision-induced dissociation of the peptide to create corresponding fragment ions. The spectrum of the fragment ions was analyzed.

Propeptide type IIB NH₂-terminal peptide fragments (PIIBNP) were first isolated from a sample of urine from a twelve-year old child. The choice to use a biological sample from a child for detection was made because the rate of synthesis of collagen type II is typically much greater in children than in healthy

adults, thereby providing a stronger signal in the mass spectrum. The mass spectrum of the sequence QDVRQPGPKGQKGEPGDIKD (SEQ ID NO. 9) of PIIBNP is shown in FIG. 3.

Post-Translational Modifications

Lysine may be converted to allysine (and hydroxylysine may be converted to hydroxyallysine) *in vivo* by lysyl oxidase by way of the following formula:



This post-translational modification was found in several PIIBNP characterized. Such post-translational modifications are intended to be within the scope of the appended claims. For example, SEQ ID NO. 9 was detected in the following modified forms:



FIG 4 shows the mass spectrum of example (4), with three hexose sugar modifications.

Six other PIIBNP were characterized and quantified in human urine, as follows:

QDVRQPGPKGQKGEPGD (SEQ ID NO. 7); QDVRQPGPKGQKGEPGDIK (SEQ ID NO. 8); QDVRQPGPKGQKGEPGDIKD (SEQ ID NO. 9); QDVRQPGPKGQKGEPGDIKDIVGPKGPPGP (SEQ ID NO. 10); QDVRQPGPKGQKGEPGDIKDIVGPKGPPGPQGPAG (SEQ ID NO. 11); and QDVRQPGPKGQKGEPGDIKDIVGPKGPPGPQGPAGEQGPRG (SEQ ID NO. 12). Of these peptides, QDVRQPGPKGQKGEPGD (SEQ ID NO. 7) and QDVRQPGPKGQKGEPGDIKD (SEQ ID NO. 9) were most abundant, in both normal and osteoarthritis patients.

Example III – Detection of PIIBNP in Osteoarthritis Patients

Urine samples were collected from twelve patients with diagnosed osteoarthritis, and six age-matched, healthy control subjects. LC/MS/MS analysis of the samples was performed as described herein. The samples were further analyzed for detection of collagen type II degradation peptides, as

previously described. Patients one through twelve had been previously diagnosed with osteoarthritis, while patients thirteen through eighteen were age-matched, healthy controls. FIG. 5 graphically depicts the concentration of collagen type II 45 mer degradation peptide present in the samples. The osteoarthritis group had a mean concentration of 6.6 ng/ml collagen type II 45 mer degradation peptide, compared with the control group with a mean concentration of 3.4 ng/ml. When the ratio of collagen type II 45 mer degradation peptide to PIIBNP was calculated, however, an even greater differential between osteoarthritis patients and normal control patients was observed. FIG. 6 graphically depicts the ratio. The osteoarthritis group had a mean ratio of 10, while the control group had a mean ratio of 4.8.

Arthritis scores for each of the twelve osteoarthritis patients were assessed, and compared to the ratios for each of the twelve osteoarthritis patients. FIG. 7 graphically shows the results of each patients arthritis score and ratio of collagen type II 45 mer degradation peptide to PIIBNP. As can be seen, there is correlation between arthritis score and collagen type II 45 mer degradation peptide to PIIBNP ratio.

Example IV- ELISA ASSAYS

1. Capture ELISA Assay

A. Selection of a First Monoclonal Antibody

A first monoclonal antibody to be used as a detection antibody is isolated or selected from known, available monoclonal antibodies such that the monoclonal antibody will demonstrate binding to a particular degradation product (a PIIBNP fragment, for example) but no significant binding to intact collagen. The generation and characterization of such a monoclonal antibody is well known in the art.

Monoclonal antibodies can be of any class of antibody, for example, IgG, IgA, IgD, IgE, and IgM.

B. Selection of a Second Monoclonal Antibody

A second monoclonal antibody to be used as a capture antibody is isolated or selected from known, available monoclonal antibodies such that the monoclonal antibody will demonstrate binding to synthesis products (PIIBNP fragments) but no significant binding to intact collagen. The generation and characterization of such a monoclonal antibody is well known in the art.

C. Sandwich Assay Using a Capture Antibody (CAb) and a Monoclonal Antibody as the Detection Antibody (Dab) for Determining Concentration of a Degradation Product in a Biological Sample.

The second monoclonal antibody of part B, above (the capture antibody, CAb) may be added to Nunc Maxisorp (VWR, Boston, Mass.) 96-well plates or their equivalent, with CAb at 10 µg/mL in 0.05M sodium borate buffer, pH 8.5 using 100 µL/well (except for control wells numbered 4, 5 and 6, see Table 1) and incubated for about 18-48 hours at 4° C.

The plate may be washed, for example, for three times with DPBS with 0.05% TW-20 (Sigma),

(DPBS/TW-20); 200 μ L/well may be used.

Wells in the plate are blocked with 1% non-fat dry milk (NFDM) dissolved in DPBS (NFDM DPBS) prepared freshly, that is, held on ice for no more than the day of use, using 100 μ L/well incubated for 1 hour at RT.

5 The blocking solution is discarded, the wells rinsed one time with 200 μ L of DPBS/TW-20.

A peptide, for example corresponding in amino acid sequence to SEQ ID NO 7, is diluted in 0.1% NFDM DPBS to concentrations shown in Table 1. The exemplary peptide has the sequence: Gln-Asp-Val-Arg-Gln-Pro-Gly-Pro-Lys-Gly-Gln-Lys-Gly-Glu-Pro-Gly-Asp, as set forth in the Sequence Listings as SEQ ID NO: 7 and may be synthesized and purified by any custom protein synthesis laboratory, such as

10 Anaspec Inc (San Jose, Calif.), for example.

The dilutions of this exemplary peptide of SEQ ID NO: 7, the samples at appropriate dilutions, and the controls are placed into the specified wells of the microtiter plate as shown in Table 1.

TABLE 1

15

Outline of a microtiter plate and antibody coating scheme

Peptide (SEQ ID NO 7) ng/mL

peptid e	peptid e	peptid e	peptid e	peptid e	peptid e	peptid e	peptid e	peptid e	peptid e	peptid e	peptid e
2	1.33	0.889	0.59	0.4	0.26	0.18	0.12	0.08	0.05	0.03	0.02_
"	"	"	"	"	"	"	"	"	"	"	"
sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"
control s	control s	control s	control s	control s	control s	control s	control s	control s	control s	control s	control s
1	1	2	2	3	3	4	4	5	5	6	6

20

TABLE 2

Additions to the control wells

<u>Controls:</u>		Biotinylated		Anti-biotin antibody
CAb	peptide	DAb	HRP-labelled	
1	+	-	-	+
2	+	+	-	+
3	+	-	+	+
4	-	+	+	+
5	-	+	-	+
6	-	-	+	+

The wells are washed three times with 200 μ L/well of DPBS TW-20.

Biotin-conjugated DAb (Bt-DAb) is added to all peptide of SEQ ID NO: 7 containing wells, all sample wells, and all control wells except 1, 2, and 5. Bt-DAb (100 μ L/well) at 1 μ g/mL in 0.1% NFDM DPBS is added to each well and the plate is incubated for 40 min at 37 °C.

Optionally, the DAb may be biotinylated using 37 μ g of biotin-N-hydroxysuccinamide (available from Pierce Chemical) per mg of DAb for 2 hrs and then dialyzed overnight using a 10 kD cut-off dialysis cassette (available from Pierce Chemical).

The wells are washed three times with 200 μ L/well of DPBS TW-20.

Mouse monoclonal anti-biotin antibody conjugated with HRP (available from Jackson ImmunoResearch) is diluted 1/5000 in 0.1% NFDM DPBS and 100 μ L/well is added to all wells and incubated for 30 minutes at RT.

The wells are washed three times with 200 μ L/well with DPBS TW-20.

100 μ L/well of 1-step Turbo (ready to use 3,3',5,5'-tetramethyl benzidine; available from Pierce Chemical) is added to each well and incubated at RT for approximately 10 minutes. Color development may be stopped with 2N H₂ SO₄. The results are read on a spectrophotometer at 450 nm.

From the concentrations of a peptide of SEQ. ID NO. 7 and the resulting optical density reading at 450 nm, a standard curve can be constructed. Over the linear portion of the curve, a regression line is used to fit the data. For samples that fall outside of the linear portion, the concentrations can be read off the graph or the samples may be diluted to fall within the standard portion of the curve, or they may be below the limit of detection. The regression between log (nM) and OD₄₅₀ gives a slope OD₄₅₀/log(nM) and an intercept OD₄₅₀. When unknown samples are run, the calibration curve can be used to determine of concentration of PIIBNP fragments from the optical density of the sample. The following calculation can be used to determine the concentration of the degradation fragment in a biological sample:

$$\text{Log(Concentration[nM])} = (\text{Sample OD}_{450} - \text{Intercept}) / \text{Slope}$$

Thus, the inverse log of the resulting number provides the concentration in nM.

Other appropriate peptides or collagen fragments can be substituted to prepare a standard curve. The units are expressed in terms of molar equivalents of standard.

2. Competitive ELISA Assay

This assay is a competitive ELISA using a 96 well format. The ELISA plate is coated overnight at room temperature with chicken IgY antibody against procollagen II N-terminal propeptide (PIINP) sequence CZGPKGQKGEPGDIKDI (SEQ ID NO. 13). The unbound antibody is removed. A fixed amount of biotinylated PIINP peptide is allowed to compete with standard peptide or unknown samples for 2 hours. Unbound PIINP is removed and biotinylated peptide is detected with Horseradish peroxidase conjugated anti-biotin and developed using Tetramethylbenzidine (TMB) substrate. This assay can be used to detect NP11 levels in human, rat, and dog serum, plasma, urine and synovial fluid, since these species share identical homology in the propeptide sequence GPKGQKGEPGDIKDI (SEQ ID NO. 13). SEQ ID NO. 14 is the full-length rat (*rattus norvegicus*) unprocessed collagen type II alpha 1 sequence. SEQ ID NO. 15 is the procollagen IIA sequence for dog (*Cannis familiaris*). SEQ ID NO. 16 is a fragment of the procollagen IIB sequence for dog (*Cannis familiaris*). FIG. 8 shows the format of this assay.

3. Competitive Solution Phase Assay

Wells of 96-well plates are coated with protein antigen (2 mg/ml in carbonate buffer, 0.13 ml/well), and kept at 4°C overnight.

Wells are aspirated and blocked with 2% BSA in TBS (250 ml/well) for 2 hours at room temperature.

Using, for example, polypropylene tubes, the antigen is diluted into antibody solution (varying antigen concentration with constant antibody). Optimum concentration of the antibody must be routinely determined.

Both antibody and antigen dilutions are conveniently made from stock solutions for which the protein concentrations are determined by absorbance measurement at 280 nm, for example. Typically, an antibody concentration of 0.5 nM and a starting antigen concentration of 10 nM is appropriate.

Serial dilutions are made into antibody diluted to the appropriate concentration in 0.1% BSA-TBS. Following a three-hour incubation at room temperature, transfer about 0.1 ml of antigen-antibody solution to duplicate or triplicate wells of antigen-coated 96-well plates, and let the plate sit at room temperature for about thirty minutes. Wells should be washed, about four times, with TBS.

Peroxidase-conjugated secondary antibody is added, and incubated for about thirty minutes at room temperature. Wells should again be washed, about four times, with TBS.

A chromogenic substrate (such as, for example, o-Phenylenediamine) is then added. The reaction is allowed to develop for about five to ten minutes, and stopped by adding 50 ml of 4N H₂SO₄, for example.

The absorbance of the wells is read at an appropriate wavelength, such as, for example, between 450-490 nm. Absorbances are averaged from duplicate wells and Absorbance vs. log [Inhibitor] is plotted.

D. Comparison of Sample Concentration to Known Standard Concentration

The concentration calculated from the biological sample is compared with a standard. The standard is preferably provided as adjusted based upon normal function, that is to say, with little synthesis

and degradation product concentration. A standard error is taken into account for the approximate standard concentration. Values outside of the standard error are considered to be abnormal.

Example V - Determination of Efficacy of Agents or Drugs that Prevent Cartilage Degradation

The present invention may be used to determine the efficacy of agents or drugs administered to treat or prevent a cartilage type II degradation mediated disorder, such as, for example, arthritis. To this end, in a first detection step, PIIBNP fragments, such as SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, or SEQ ID NO. 12, or combinations thereof, are detected in a biological sample obtained from an individual exhibiting the signs and symptoms of arthritis, or an individual predisposed to an arthritis condition. Collagen type II degradation fragments may also be detected in a biological sample obtained from the same individual, as previously described. The biological sample may be the same sample, or a different sample. Optionally, both PIIBNP fragments and collagen type II degradation fragments are both obtained from the same individual. In this optional step, the ratio of PIIBNP fragments to collagen type II degradation fragments may be compared, and this ratio may be used to determine the presence or stage of arthritis. An agent or drug intended to decrease or inhibit a collagenase is administered to the individual. A subsequent, second detection step is performed, in which detection of PIIBNP fragments is carried out at a time after administration of the agent or drug intended to decrease or inhibit a collagenase, and optionally, detection of collagen type II degradation fragments is also performed. The presence, and optionally the quantity, of PIIBNP fragments, and optionally the presence and quantity of collagen type II degradation fragments is compared with the results of the first detection step, and the efficacy of the agent or drug administered to decrease or inhibit the collagenase is determined based upon the resulting detection.

The following non-limiting list of individual patents and publications describe MMP inhibitors useful in the practice of the present invention: U.S. Patent No. 6,541,489; U.S. Patent No. 6,448,250; WO 00/69821; WO 02/064599; US2002-0156061; US2003-0004172; U.S. published application 2003-0078276; U.S. published application 2003-0130278; U.S. published application 2002-0193377; U.S. published application 2002-0151558; U.S. published application 2002-0156069; U.S. published application 2002-0151555; U.S. published application 2002-0161000; U.S. published application 2003-0087924; U.S. published application 2003-0144274; WO 02/064080; WO 02/064568; WO 02/064571; WO 02/064572; WO 02/064578; WO 02/064595; WO 02/064598; WO 03/032999; WO 02/064547; WO 03/076417; WO 03/033478 and WO 04/000811.

In particular, the following MMP inhibitors would be useful in the practice of the present invention: N-hydroxy-4-({4-[5-(3,3,4,4,4-pentafluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; N-hydroxy-4-({4-[5-(4,4,4-trifluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; N-hydroxy-4-({4-[5-(3,3,3-trifluoropropyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; 1-(2-methoxyethyl)-4-({4-[5-(4,4,4-trifluorobutyl)(2-pyridyl)]phenyl}sulfonyl)piperidine-4-carboxylic acid, dihydrochloride; 4-({4-[5-(2-cyclopropylethyl)pyrazin-2-yl]phenyl}sulfonyl)-N-hydroxytetrahydro-2H-pyran-4-carboxamide hydrochloride; 4-({4-[5-(3,3-difluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)-N-hydroxytetrahydro-2H-pyran-4-carboxamide hydrochloride; 4-[6-(4-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-

- d]pyrimidin-3-ylmethyl]-benzoic acid; 4-{4-oxo-6-[(pyridin-3-ylmethyl)-carbamoyl]-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-chloro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-{4-oxo-6-[(pyridin-4-ylmethyl)-carbamoyl]-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl}-benzoic acid; 4-{6-[(2-methoxy-pyridin-4-ylmethyl)-carbamoyl]-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl}-benzoic acid; 4-[6-(4-methylsulfanyl-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-fluoro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-benzylcarbamoyl]-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-chloro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-fluoro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-(4-trifluoromethyl-benzylcarbamoyl)-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-(3-trifluoromethyl-benzylcarbamoyl)-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3,4-difluoro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-hydroxy-3-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid;
- 4-[[4-(4-bromophenyl)-4-hydroxy-1-piperidiny]sulfonyl]-tetrahydro-N-hydroxy-2H-pyran-4-carboxamide; and 4-[6-(4-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-cyclohexanecarboxylic acid.

CLAIMS

What is claimed is:

- 5 1. A method of diagnosing a condition mediated by the pathological degradation of collagen type II in a subject comprising:
 - detecting at least one PIIBNP in a biological sample obtained from said subject; and quantifying the concentration of said PIIBNP from said biological sample.
- 10 2. The method of claim 1 wherein said PIIBNP is selected from the group consisting of SEQ ID 9, SEQ ID 10, SEQ ID 11, and SEQ ID 12.
3. The method of claim 1 wherein a plurality of PIINP are collected.
- 15 4. The method of claim 1 further comprising detecting at least one degradation peptide of collagen type II, quantifying the concentration of said degradation peptide of collagen type II, and determining the ratio of said concentration of said degradation peptide of collagen type II to said concentration of said PIIBNP.
- 20 5. The method of claim 4 wherein said biological sample is urine.
6. The method of claim 4 wherein said biological sample is collected contemporaneously.
7. The method of claim 6 wherein said biological sample is collected in a single vessel.
- 25 8. The method of claim 1 wherein said biological sample obtained from said subject represent a first sample, and second biological sample is collected from said subject at a subsequent time, and at least one PIIBNP in a biological sample is obtained from said subject from said second sample; the concentration of said PIIBNP from said second biological sample is quantified, and said concentration of

30 said PIIBNP from said second sample is compared with the concentration of said first sample.
9. A method of determining the efficacy of an agent or drug intended to decrease or inhibit a collagenase enzyme comprising:
 - obtaining a first biological sample from a subject with a collagen type II degradation mediated

35 disorder;
 - detecting a PIIBNP in said first biological sample;
 - quantifying the concentration of said PIIBNP in said first biological sample,
 - detecting a collagen type II degradation peptide in said first biological sample;
 - quantifying the concentration of said collagen type II degradation peptide;
 - 40 administering an agent or drug intended to decrease or inhibit a collagenase enzyme;
 - obtaining a second biological sample from said subject;

quantifying the concentration of said PIIBNP in said second biological sample,
 detecting a collagen type II degradation peptide in said second biological sample;
 quantifying the concentration of said collagen type II degradation peptide; and
 comparing the concentration of said PIIBNP and said concentration of said collagen type II degradation
 5 peptide from said second sample with the concentration of said PIIBNP and said concentration of said
 collagen type II degradation peptide from said first sample to determine the efficacy of said agent or drug
 intended to decrease or inhibit a collagenase.

10. The method of claim 9 wherein said agent or drug intended to decrease or inhibit a collagenase is
 10 selected from the group consisting of:

N-hydroxy-4-({4-[5-(3,3,4,4,4-pentafluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-
 carboxamide hydrochloride; N-hydroxy-4-({4-[5-(4,4,4-trifluorobutyl)pyrazin-2-
 yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; N-hydroxy-4-({4-[5-(3,3,3-
 trifluoropropyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; 1-(2-
 15 methoxyethyl)-4-({4-[5-(4,4,4-trifluorobutyl)(2-pyridyl)]phenyl}sulfonyl)piperidine-4-carboxylic acid,
 dihydrochloride; 4-({4-[5-(2-cyclopropylethyl)pyrazin-2-yl]phenyl}sulfonyl)-N-hydroxytetrahydro-2H-
 pyran-4-carboxamide hydrochloride; 4-({4-[5-(3,3-difluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)-N-
 hydroxytetrahydro-2H-pyran-4-carboxamide hydrochloride; 4-[6-(4-methoxy-benzylcarbamoyl)-4-oxo-4H-
 pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-
 20 d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-[(pyridin-3-ylmethyl)-carbamoyl]-4H-pyrido[3,4-
 d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-chloro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-
 ylmethyl]-benzoic acid; 4-[4-oxo-6-[(pyridin-4-ylmethyl)-carbamoyl]-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-
 benzoic acid; 4-[6-[(2-methoxy-pyridin-4-ylmethyl)-carbamoyl]-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-
 25 ylmethyl]-benzoic acid; 4-[6-(4-fluoro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-
 benzoic acid; 4-[6-benzylcarbamoyl]-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-
 chloro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-fluoro-
 benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-(4-trifluoromethyl-
 benzylcarbamoyl)-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-(3-trifluoromethyl-
 30 benzylcarbamoyl)-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3,4-difluoro-
 benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-hydroxy-3-methoxy-
 benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid;
 4-[[4-(4-bromophenyl)-4-hydroxy-1-piperidinyl]sulfonyl]-tetrahydro-N-hydroxy-2H-pyran-4-
carboxamide; and 4-[6-(4-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido-[3,4-d]pyrimidin-3-ylmethyl]-
 35 cyclohexanecarboxylic acid.

11. A method of determining the an appropriate dose of an agent or drug intended to decrease or
 inhibit a collagenase enzyme comprising:

obtaining a first biological sample from a subject with a collagen type II degradation mediated
 40 disorder;
 detecting a PIIBNP in said first biological sample;

- quantifying the concentration of said PIIBNP in said first biological sample,
 detecting a collagen type II degradation peptide in said first biological sample;
 quantifying the concentration of said collagen type II degradation peptide;
 administering an agent or drug intended to decrease or inhibit a collagenase enzyme in a fixed
 5 dose;
 obtaining a second biological sample from said subject;
 quantifying the concentration of said PIIBNP in said second biological sample,
 detecting a collagen type II degradation peptide in said second biological sample;
 quantifying the concentration of said collagen type II degradation peptide; and
 10 comparing the concentration of said PIIBNP and said concentration of said collagen type II degradation
 peptide from said second sample with the concentration of said PIIBNP and said concentration of said
 collagen type II degradation peptide from said first sample to determine the appropriate dose of said
 agent or drug intended to decrease or inhibit a collagenase.
- 15 12. The method of claim 11 wherein said agent or drug intended to decrease or inhibit a collagenase is
 selected from the group consisting of:
 N-hydroxy-4-({4-[5-(3,3,4,4,4-pentafluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-
 carboxamide hydrochloride; N-hydroxy-4-({4-[5-(4,4,4-trifluorobutyl)pyrazin-2-
 20 yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; N-hydroxy-4-({4-[5-(3,3,3-
 trifluoropropyl)pyrazin-2-yl]phenyl}sulfonyl)tetrahydro-2H-pyran-4-carboxamide hydrochloride; 1-(2-
 methoxyethyl)-4-({4-[5-(4,4,4-trifluorobutyl)(2-pyridyl)]phenyl}sulfonyl)piperidine-4-carboxylic acid,
 dihydrochloride; 4-({4-[5-(2-cyclopropylethyl)pyrazin-2-yl]phenyl}sulfonyl)-N-hydroxytetrahydro-2H-
 pyran-4-carboxamide hydrochloride; 4-({4-[5-(3,3-difluorobutyl)pyrazin-2-yl]phenyl}sulfonyl)-N-
 hydroxytetrahydro-2H-pyran-4-carboxamide hydrochloride; 4-[6-(4-methoxy-benzylcarbamoyl)-4-oxo-4H-
 25 pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-
 d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-[(pyridin-3-ylmethyl)-carbamoyl]-4H-pyrido[3,4-
 d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-chloro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-
 ylmethyl]-benzoic acid; 4-[4-oxo-6-[(pyridin-4-ylmethyl)-carbamoyl]-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-
 benzoic acid; 4-[6-[(2-methoxy-pyridin-4-ylmethyl)-carbamoyl]-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-
 30 ylmethyl]-benzoic acid; 4-[6-(4-methylsulfanyl-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-
 ylmethyl]-benzoic acid; 4-[6-(4-fluoro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-
 benzoic acid; 4-[6-benzylcarbamoyl]-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-
 chloro-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3-fluoro-
 benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-(4-trifluoromethyl-
 35 benzylcarbamoyl)-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[4-oxo-6-(3-trifluoromethyl-
 benzylcarbamoyl)-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(3,4-difluoro-
 benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid; 4-[6-(4-hydroxy-3-methoxy-
 benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-benzoic acid;
 4-[4-(4-bromophenyl)-4-hydroxy-1-piperidinyl]sulfonyl-tetrahydro-N-hydroxy-2H-pyran-4-
 40 carboxamide; and 4-[6-(4-methoxy-benzylcarbamoyl)-4-oxo-4H-pyrido[3,4-d]pyrimidin-3-ylmethyl]-
 cyclohexanecarboxylic acid.

13. A peptide, or post-translationally modified or derivitized peptide selected from the group consisting of:

SEQ ID NO. 9, SEQ ID NO 10; SEQ ID NO. 11 and SEQ ID NO. 12.

5

14. The use of a peptide, or post-translationally modified or derivitized peptide selected from the group consisting of:

SEQ ID NO. 9, SEQ ID NO 10; SEQ ID NO. 11 and SEQ ID NO. 12, as a biomarker of a collagen type II degradation mediated disorder.

10

Performance of CZGPKGQKGEPGDIKDI IgY Column

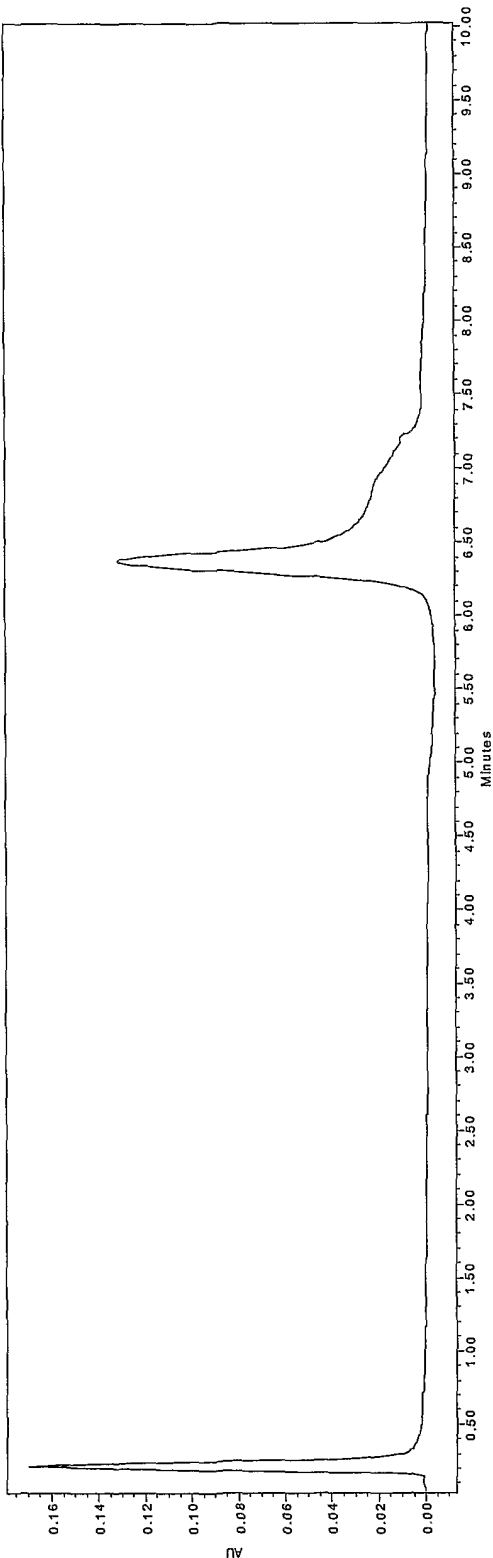


FIG. 1

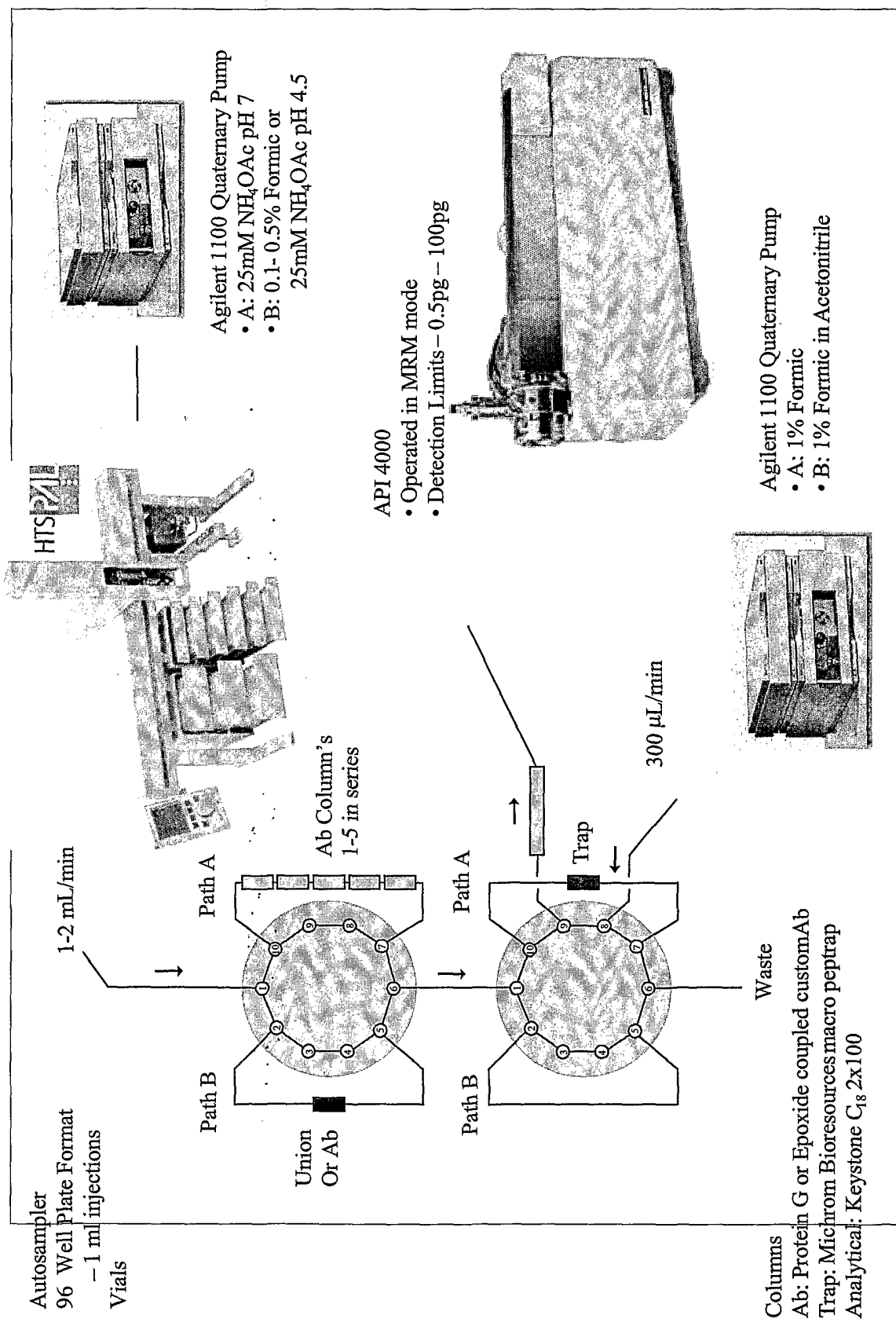


FIG 2

**LC/MS Spectrum of QDVRQPGPKGQKGEPGDIKD
Isolated from Child Urine via N-Procollagen Type II Ab
Column**

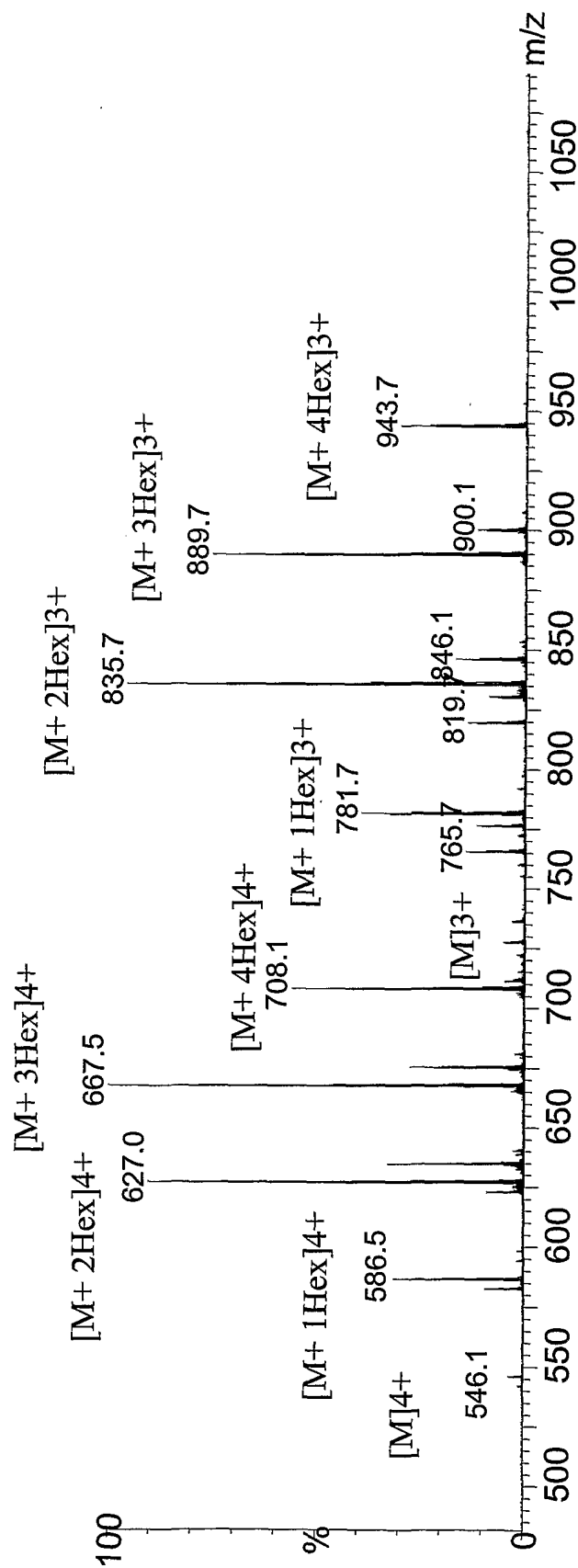


FIG 3

**MS/MS Spectrum of QDVRQPGP(aHyK)GQKGEPGDIKD,
m/z 884.3, with 3 Hexose**

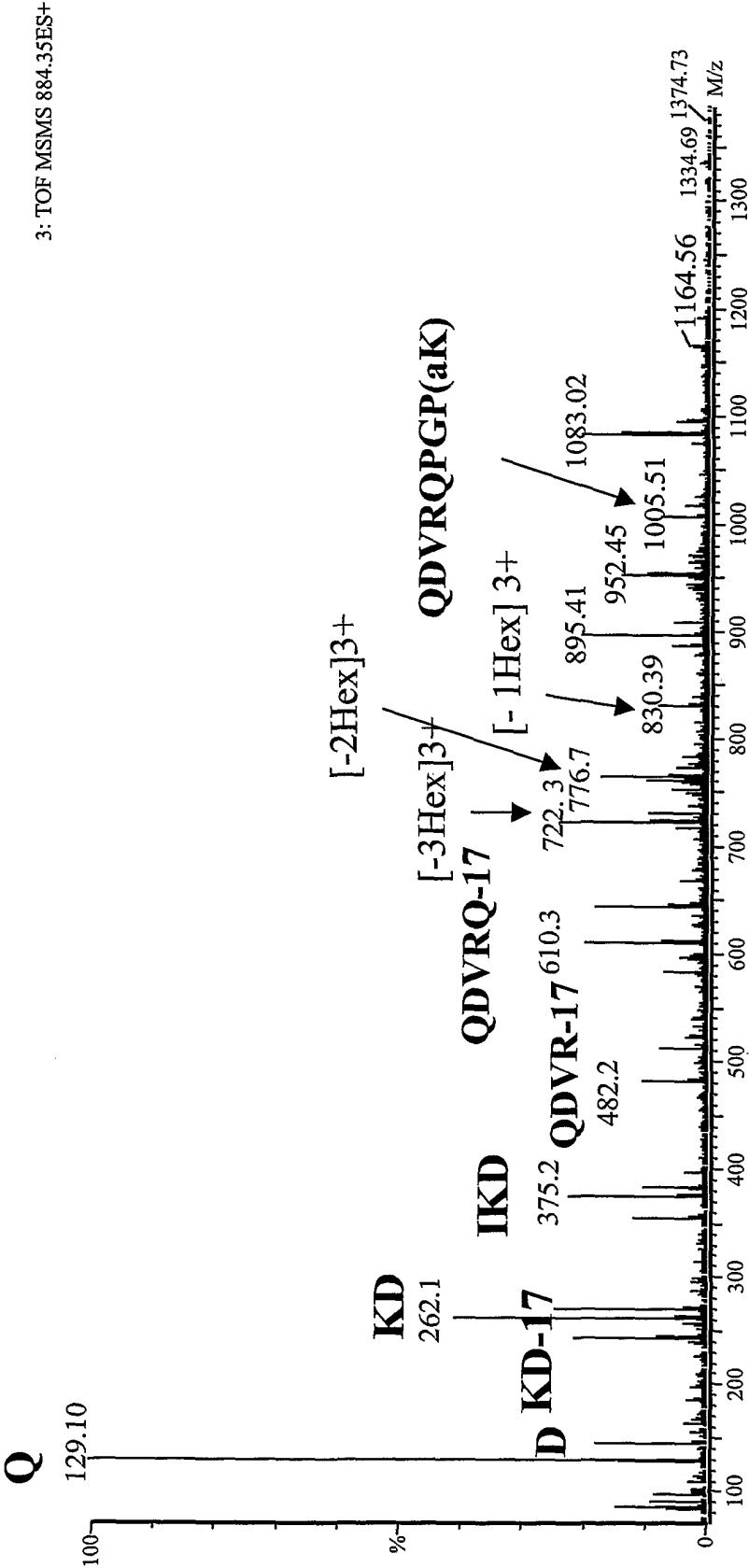


FIG 4

Level of Collagen Type II Peptides in Urine Samples

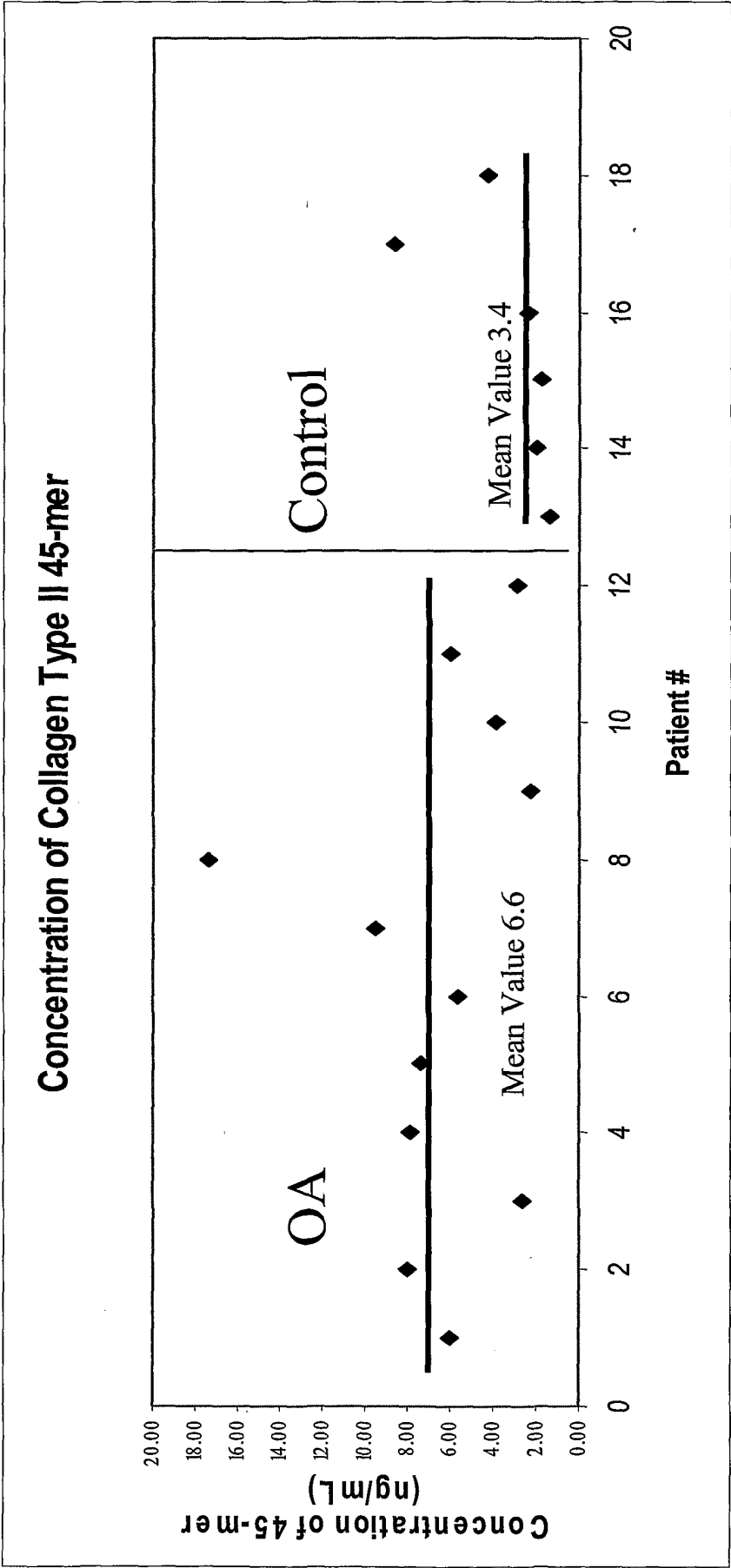


FIG 5

Ratios of Collagen II Degradation Peptides /Pro (LC-MS/MS)

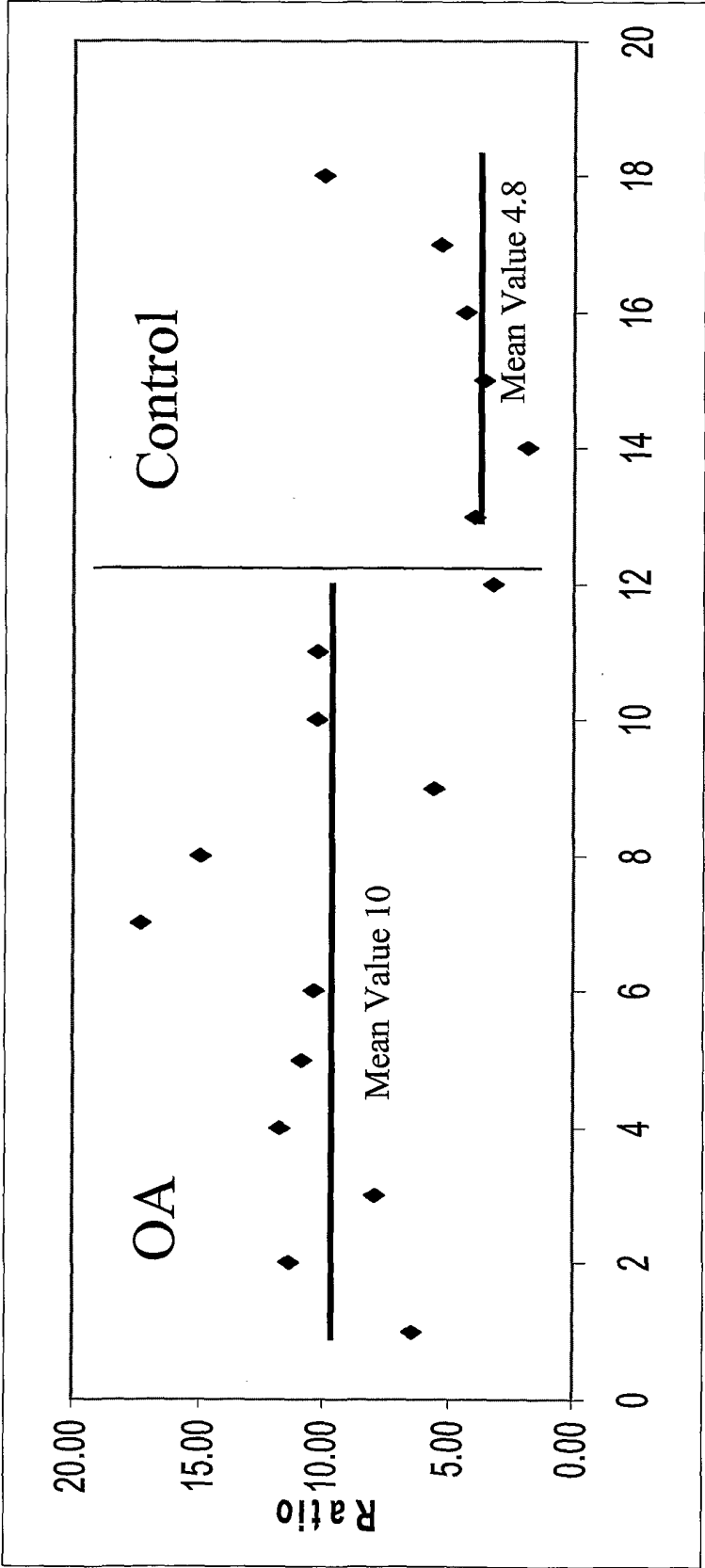


FIG 6

Correlation of Ratios of Collagen II Degradation Peptides /Pro (LC-MS/MS) to Total Arthritis Score

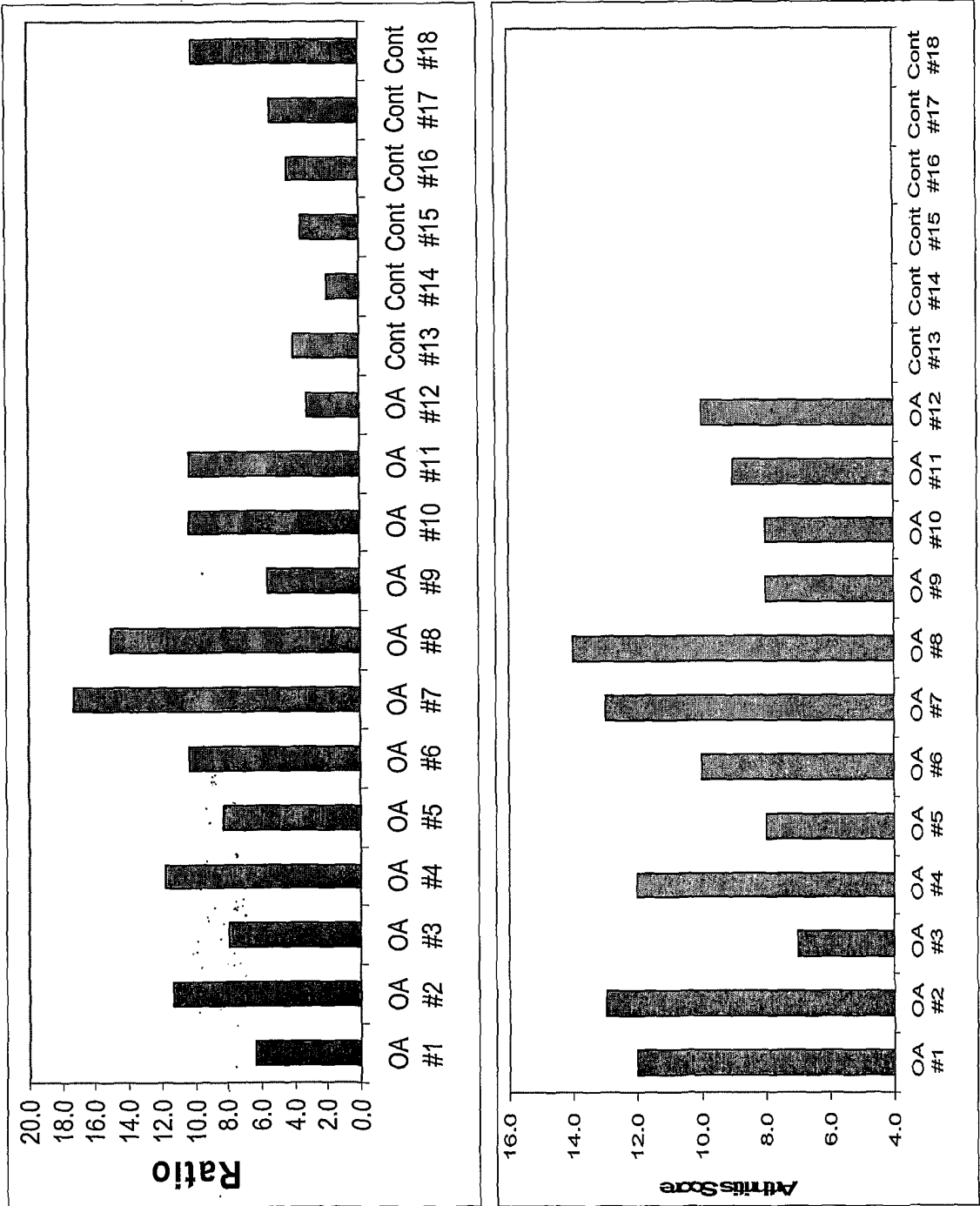


FIG. 7.

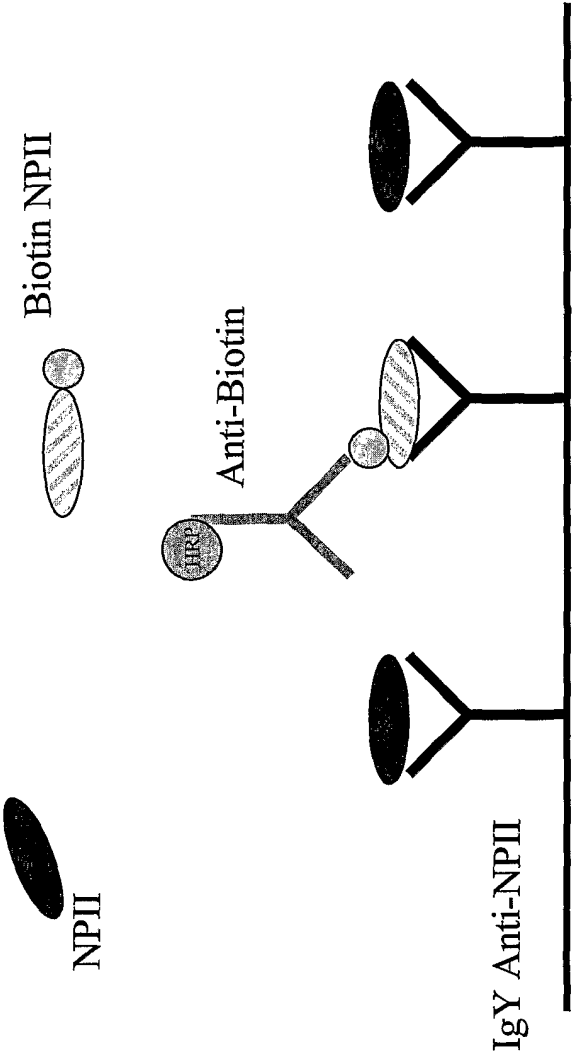


FIG 8

SEQ listing 32350.ST25
SEQUENCE LISTING

<110> Nemirovskiy, Olga

<120> Procollagen Biomarkers and Methods

<130> PC-XXX

<160> 16

<170> PatentIn version 3.2

<210> 1

<211> 1418

<212> PRT

<213> Homo sapiens

<400> 1

Met Ile Arg Leu Gly Ala Pro Gln Ser Leu Val Leu Leu Thr Leu Leu
1 5 10 15

Val Ala Ala Val Leu Arg Cys Gln Gly Gln Asp Val Arg Gln Pro Gly
20 25 30

Pro Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile Val Gly
35 40 45

Pro Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala Gly Glu Gln Gly Pro
50 55 60

Arg Gly Asp Arg Gly Asp Lys Gly Glu Lys Gly Ala Pro Gly Pro Arg
65 70 75 80

Gly Arg Asp Gly Glu Pro Gly Thr Leu Gly Asn Pro Gly Pro Pro Gly
85 90 95

Pro Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala
100 105 110

Gln Met Ala Gly Gly Phe Asp Glu Lys Ala Gly Gly Ala Gln Leu Gly
115 120 125

Val Met Gln Gly Pro Met Gly Pro Met Gly Pro Arg Gly Pro Pro Gly
130 135 140

Pro Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Asn Pro Gly Glu
145 150 155 160

Pro Gly Glu Pro Gly Val Ser Gly Pro Met Gly Pro Arg Gly Pro Pro
165 170 175

SEQ listing 32350.ST25

Gly Pro Pro Gly Lys Pro Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly
 180 185 190

Lys Ala Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Phe
 195 200 205

Pro Gly Thr Pro Gly Leu Pro Gly Val Lys Gly His Arg Gly Tyr Pro
 210 215 220

Gly Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala Pro Gly Val Lys Gly
 225 230 235 240

Glu Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro Gly Pro Met Gly Pro
 245 250 255

Arg Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly Pro Ala Gly Ala Ala
 260 265 270

Gly Ala Arg Gly Asn Asp Gly Gln Pro Gly Pro Ala Gly Pro Pro Gly
 275 280 285

Pro Val Gly Pro Ala Gly Gly Pro Gly Phe Pro Gly Ala Pro Gly Ala
 290 295 300

Lys Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Ala Gln
 305 310 315 320

Gly Pro Arg Gly Glu Pro Gly Thr Pro Gly Ser Pro Gly Pro Ala Gly
 325 330 335

Ala Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro Gly Ala Lys Gly Ser
 340 345 350

Ala Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Pro Arg
 355 360 365

Gly Pro Pro Asp Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys Gly
 370 375 380

Gln Thr Gly Lys Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro
 385 390 395 400

Lys Gly Glu Pro Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro Ala
 405 410 415

Gly Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Gly Val Gly
 420 425 430

SEQ listing 32350.ST25

Pro Ile Gly Pro Pro Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly Phe
 435 440 445
 Pro Gly Gln Asp Gly Leu Ala Gly Pro Lys Gly Ala Pro Gly Glu Arg
 450 455 460
 Gly Pro Ser Gly Leu Ala Gly Pro Lys Gly Ala Asn Gly Asp Pro Gly
 465 470 475 480
 Arg Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly Arg
 485 490 495
 Pro Gly Asp Ala Gly Pro Gln Gly Lys Val Gly Pro Ser Gly Ala Pro
 500 505 510
 Gly Glu Asp Gly Arg Pro Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly
 515 520 525
 Gln Pro Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Asn Gly Glu
 530 535 540
 Pro Gly Lys Ala Gly Glu Lys Gly Leu Pro Gly Ala Pro Gly Leu Arg
 545 550 555 560
 Gly Leu Pro Gly Lys Asp Gly Glu Thr Gly Ala Glu Gly Pro Pro Gly
 565 570 575
 Pro Ala Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly Ala Pro Gly Pro
 580 585 590
 Ser Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Glu Gly
 595 600 605
 Gly Lys Pro Gly Asp Gln Gly Val Pro Gly Glu Ala Gly Ala Pro Gly
 610 615 620
 Leu Val Gly Pro Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Ser
 625 630 635 640
 Pro Gly Ala Gln Gly Leu Gln Gly Pro Arg Gly Leu Pro Gly Thr Pro
 645 650 655
 Gly Thr Asp Gly Pro Lys Gly Ala Ser Gly Pro Ala Gly Pro Pro Gly
 660 665 670
 Ala Gln Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala
 675 680 685

SEQ listing 32350.ST25

Ala Gly Ile Ala Gly Pro Lys Gly Asp Arg Gly Asp Val Gly Glu Lys
 690 695 700
 Gly Pro Glu Gly Ala Pro Gly Lys Asp Gly Gly Arg Gly Leu Thr Gly
 705 710 715 720
 Pro Ile Gly Pro Pro Gly Pro Ala Gly Ala Asn Gly Glu Lys Gly Glu
 725 730 735
 Val Gly Pro Pro Gly Pro Ala Gly Ser Ala Gly Ala Arg Gly Ala Pro
 740 745 750
 Gly Glu Arg Gly Glu Thr Gly Pro Pro Gly Thr Ser Gly Ile Ala Gly
 755 760 765
 Pro Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Gln Gly Glu
 770 775 780
 Ala Gly Gln Lys Gly Asp Ala Gly Ala Pro Gly Pro Gln Gly Pro Ser
 785 790 795 800
 Gly Ala Pro Gly Pro Gln Gly Pro Thr Gly Val Thr Gly Pro Lys Gly
 805 810 815
 Ala Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala
 820 825 830
 Ala Gly Arg Val Gly Pro Pro Gly Ser Asn Gly Asn Pro Gly Pro Pro
 835 840 845
 Gly Pro Pro Gly Pro Ser Gly Lys Asp Gly Pro Lys Gly Ala Arg Gly
 850 855 860
 Asp Ser Gly Pro Pro Gly Arg Ala Gly Glu Pro Gly Leu Gln Gly Pro
 865 870 875 880
 Ala Gly Pro Pro Gly Glu Lys Gly Glu Pro Gly Asp Asp Gly Pro Ser
 885 890 895
 Gly Ala Glu Gly Pro Pro Gly Pro Gln Gly Leu Ala Gly Gln Arg Gly
 900 905 910
 Ile Val Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu
 915 920 925
 Pro Gly Pro Ser Gly Glu Pro Gly Gln Gln Gly Ala Pro Gly Ala Ser
 Page 4

SEQ Listing 32350.ST25

930 935 940
 Gly Asp Arg Gly Pro Pro Gly Pro Val Gly Pro Pro Gly Leu Thr Gly
 945 950 955 960
 Pro Ala Gly Glu Pro Gly Arg Glu Gly Ser Pro Gly Ala Asp Gly Pro
 965 970 975
 Pro Gly Arg Asp Gly Ala Ala Gly Val Lys Gly Asp Arg Gly Glu Thr
 980 985 990
 Gly Ala Val Gly Ala Pro Gly Ala Pro Gly Pro Pro Gly Ser Pro Gly
 995 1000 1005
 Pro Ala Gly Pro Thr Gly Lys Gln Gly Asp Arg Gly Glu Ala Gly
 1010 1015 1020
 Ala Gln Gly Pro Met Gly Pro Ser Gly Pro Ala Gly Ala Arg Gly
 1025 1030 1035
 Ile Gln Gly Pro Gln Gly Pro Arg Gly Asp Lys Gly Glu Ala Gly
 1040 1045 1050
 Glu Pro Gly Glu Arg Gly Leu Lys Gly His Arg Gly Phe Thr Gly
 1055 1060 1065
 Leu Gln Gly Leu Pro Gly Pro Pro Gly Pro Ser Gly Asp Gln Gly
 1070 1075 1080
 Ala Ser Gly Pro Ala Gly Pro Ser Gly Pro Arg Gly Pro Pro Gly
 1085 1090 1095
 Pro Val Gly Pro Ser Gly Lys Asp Gly Ala Asn Gly Ile Pro Gly
 1100 1105 1110
 Pro Ile Gly Pro Pro Gly Pro Arg Gly Arg Ser Gly Glu Thr Gly
 1115 1120 1125
 Pro Ala Gly Pro Pro Gly Asn Pro Gly Pro Pro Gly Pro Pro Gly
 1130 1135 1140
 Pro Pro Gly Pro Gly Ile Asp Met Ser Ala Phe Ala Gly Leu Gly
 1145 1150 1155
 Pro Arg Glu Lys Gly Pro Asp Pro Leu Gln Tyr Met Arg Ala Asp
 1160 1165 1170

SEQ listing 32350.ST25

Gln Ala Ala Gly Gly Leu Arg Gln His Asp Ala Glu Val Asp Ala
 1175 1180 1185

Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Ser Ile Arg Ser Pro
 1190 1195 1200

Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys Arg Asp Leu Lys
 1205 1210 1215

Leu Cys His Pro Glu Trp Lys Ser Gly Asp Tyr Trp Ile Asp Pro
 1220 1225 1230

Asn Gln Gly Cys Thr Leu Asp Ala Met Lys Val Phe Cys Asn Met
 1235 1240 1245

Glu Thr Gly Glu Thr Cys Val Tyr Pro Asn Pro Ala Asn Val Pro
 1250 1255 1260

Lys Lys Asn Trp Trp Ser Ser Lys Ser Lys Glu Lys Lys His Ile
 1265 1270 1275

Trp Phe Gly Glu Thr Ile Asn Gly Gly Phe His Phe Ser Tyr Gly
 1280 1285 1290

Asp Asp Asn Leu Ala Pro Asn Thr Ala Asn Val Gln Met Thr Phe
 1295 1300 1305

Leu Arg Leu Leu Ser Thr Glu Gly Ser Gln Asn Ile Thr Tyr His
 1310 1315 1320

Cys Lys Asn Ser Ile Ala Tyr Leu Asp Glu Ala Ala Gly Asn Leu
 1325 1330 1335

Lys Lys Ala Leu Leu Ile Gln Gly Ser Asn Asp Val Glu Ile Arg
 1340 1345 1350

Ala Glu Gly Asn Ser Arg Phe Thr Tyr Thr Ala Leu Lys Asp Gly
 1355 1360 1365

Cys Thr Lys His Thr Gly Lys Trp Gly Lys Thr Val Ile Glu Tyr
 1370 1375 1380

Arg Ser Gln Lys Thr Ser Arg Leu Pro Ile Ile Asp Ile Ala Pro
 1385 1390 1395

Met Asp Ile Gly Gly Pro Glu Gln Glu Phe Gly Val Asp Ile Gly
 1400 1405 1410

SEQ listing 32350.ST25

Pro Val Cys Phe Leu
1415

<210> 2
<211> 25
<212> PRT
<213> Homo sapiens

<400> 2

Met Ile Arg Leu Gly Ala Pro Gln Ser Leu Val Leu Leu Thr Leu Leu
1 5 10 15

Val Ala Ala Val Leu Arg Cys Gln Gly
20 25

<210> 3
<211> 87
<212> PRT
<213> Homo sapiens

<400> 3

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
1 5 10 15

Asp Ile Lys Asp Ile Val Gly Pro Lys Gly Pro Pro Gly Pro Gln Gly
20 25 30

Pro Ala Gly Glu Gln Gly Pro Arg Gly Asp Arg Gly Asp Lys Gly Glu
35 40 45

Lys Gly Ala Pro Gly Pro Arg Gly Arg Asp Gly Glu Pro Gly Thr Leu
50 55 60

Gly Asn Pro Gly Pro Pro Gly Pro Pro Gly Pro Gly Pro Pro Gly
65 70 75 80

Leu Gly Gly Asn Phe Ala Ala
85

<210> 4
<211> 246
<212> PRT
<213> Homo sapiens

<400> 4

Asp Gln Ala Ala Gly Gly Leu Arg Gln His Asp Ala Glu Val Asp Ala
1 5 10 15

Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Ser Ile Arg Ser Pro Gly
Page 7

<210>	5
<211>	1014
<212>	PRT
<213>	Homo sapiens

SEQ listing 32350.ST25

<400> 5

Gly Pro Met Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro Ala Gly
 1 5 10 15
 Ala Pro Gly Pro Gln Gly Phe Gln Gly Asn Pro Gly Glu Pro Gly Glu
 20 25 30
 Pro Gly Val Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro Pro
 35 40 45
 Gly Lys Pro Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Lys Ala Gly
 50 55 60
 Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Phe Pro Gly Thr
 65 70 75 80
 Pro Gly Leu Pro Gly Val Lys Gly His Arg Gly Tyr Pro Gly Leu Asp
 85 90 95
 Gly Ala Lys Gly Glu Ala Gly Ala Pro Gly Val Lys Gly Glu Ser Gly
 100 105 110
 Ser Pro Gly Glu Asn Gly Ser Pro Gly Pro Met Gly Pro Arg Gly Leu
 115 120 125
 Pro Gly Glu Arg Gly Arg Thr Gly Pro Ala Gly Ala Ala Gly Ala Arg
 130 135 140
 Gly Asn Asp Gly Gln Pro Gly Pro Ala Gly Pro Pro Gly Pro Val Gly
 145 150 155 160
 Pro Ala Gly Gly Pro Gly Phe Pro Gly Ala Pro Gly Ala Lys Gly Glu
 165 170 175
 Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Ala Gln Gly Pro Arg
 180 185 190
 Gly Glu Pro Gly Thr Pro Gly Ser Pro Gly Pro Ala Gly Ala Ser Gly
 195 200 205
 Asn Pro Gly Thr Asp Gly Ile Pro Gly Ala Lys Gly Ser Ala Gly Ala
 210 215 220
 Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Pro Arg Gly Pro Pro
 225 230 235 240

SEQ listing 32350.ST25

Asp Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys Gly Gln Thr Gly
245 250 255

Lys Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys Gly Glu
260 265 270

Pro Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro Ala Gly Glu Glu
275 280 285

Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Gly Val Gly Pro Ile Gly
290 295 300

Pro Pro Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly Phe Pro Gly Gln
305 310 315 320

Asp Gly Leu Ala Gly Pro Lys Gly Ala Pro Gly Glu Arg Gly Pro Ser
325 330 335

Gly Leu Ala Gly Pro Lys Gly Ala Asn Gly Asp Pro Gly Arg Pro Gly
340 345 350

Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly Arg Pro Gly Asp
355 360 365

Ala Gly Pro Gln Gly Lys Val Gly Pro Ser Gly Ala Pro Gly Glu Asp
370 375 380

Gly Arg Pro Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Gln Pro Gly
385 390 395 400

Val Met Gly Phe Pro Gly Pro Lys Gly Ala Asn Gly Glu Pro Gly Lys
405 410 415

Ala Gly Glu Lys Gly Leu Pro Gly Ala Pro Gly Leu Arg Gly Leu Pro
420 425 430

Gly Lys Asp Gly Glu Thr Gly Ala Glu Gly Pro Pro Gly Pro Ala Gly
435 440 445

Pro Ala Gly Glu Arg Gly Glu Gln Gly Ala Pro Gly Pro Ser Gly Phe
450 455 460

Gln Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Glu Gly Gly Lys Pro
465 470 475 480

Gly Asp Gln Gly Val Pro Gly Glu Ala Gly Ala Pro Gly Leu Val Gly
485 490 495

SEQ listing 32350.ST25

Pro Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Ser Pro Gly Ala
 500 505 510
 Gln Gly Leu Gln Gly Pro Arg Gly Leu Pro Gly Thr Pro Gly Thr Asp
 515 520 525
 Gly Pro Lys Gly Ala Ser Gly Pro Ala Gly Pro Pro Gly Ala Gln Gly
 530 535 540
 Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala Gly Ile
 545 550 555 560
 Ala Gly Pro Lys Gly Asp Arg Gly Asp Val Gly Glu Lys Gly Pro Glu
 565 570 575
 Gly Ala Pro Gly Lys Asp Gly Gly Arg Gly Leu Thr Gly Pro Ile Gly
 580 585 590
 Pro Pro Gly Pro Ala Gly Ala Asn Gly Glu Lys Gly Glu Val Gly Pro
 595 600 605
 Pro Gly Pro Ala Gly Ser Ala Gly Ala Arg Gly Ala Pro Gly Glu Arg
 610 615 620
 Gly Glu Thr Gly Pro Pro Gly Thr Ser Gly Ile Ala Gly Pro Pro Gly
 625 630 635 640
 Ala Asp Gly Gln Pro Gly Ala Lys Gly Glu Gln Gly Glu Ala Gly Gln
 645 650 655
 Lys Gly Asp Ala Gly Ala Pro Gly Pro Gln Gly Pro Ser Gly Ala Pro
 660 665 670
 Gly Pro Gln Gly Pro Thr Gly Val Thr Gly Pro Lys Gly Ala Arg Gly
 675 680 685
 Ala Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala Gly Arg
 690 695 700
 Val Gly Pro Pro Gly Ser Asn Gly Asn Pro Gly Pro Pro Gly Pro Pro
 705 710 715 720
 Gly Pro Ser Gly Lys Asp Gly Pro Lys Gly Ala Arg Gly Asp Ser Gly
 725 730 735
 Pro Pro Gly Arg Ala Gly Glu Pro Gly Leu Gln Gly Pro Ala Gly Pro
 740 745 750

SEQ listing 32350.ST25

Pro Gly Glu Lys Gly Glu Pro Gly Asp Asp Gly Pro Ser Gly Ala Glu
 755 760 765
 Gly Pro Pro Gly Pro Gln Gly Leu Ala Gly Gln Arg Gly Ile Val Gly
 770 775 780
 Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro Gly Pro
 785 790 795 800
 Ser Gly Glu Pro Gly Gln Gln Gly Ala Pro Gly Ala Ser Gly Asp Arg
 805 810 815
 Gly Pro Pro Gly Pro Val Gly Pro Pro Gly Leu Thr Gly Pro Ala Gly
 820 825 830
 Glu Pro Gly Arg Glu Gly Ser Pro Gly Ala Asp Gly Pro Pro Gly Arg
 835 840 845
 Asp Gly Ala Ala Gly Val Lys Gly Asp Arg Gly Glu Thr Gly Ala Val
 850 855 860
 Gly Ala Pro Gly Ala Pro Gly Pro Pro Gly Ser Pro Gly Pro Ala Gly
 865 870 875 880
 Pro Thr Gly Lys Gln Gly Asp Arg Gly Glu Ala Gly Ala Gln Gly Pro
 885 890 895
 Met Gly Pro Ser Gly Pro Ala Gly Ala Arg Gly Ile Gln Gly Pro Gln
 900 905 910
 Gly Pro Arg Gly Asp Lys Gly Glu Ala Gly Glu Pro Gly Glu Arg Gly
 915 920 925
 Leu Lys Gly His Arg Gly Phe Thr Gly Leu Gln Gly Leu Pro Gly Pro
 930 935 940
 Pro Gly Pro Ser Gly Asp Gln Gly Ala Ser Gly Pro Ala Gly Pro Ser
 945 950 955 960
 Gly Pro Arg Gly Pro Pro Gly Pro Val Gly Pro Ser Gly Lys Asp Gly
 965 970 975
 Ala Asn Gly Ile Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly Arg
 980 985 990
 Ser Gly Glu Thr Gly Pro Ala Gly Pro Pro Gly Asn Pro Gly Pro Pro
 Page 12

[illegible]

Gly Pro Pro Gly Pro Pro
1010

<210>	6
<211>	178
<212>	PRT
<213>	Homo sapiens
<400>	6

Gln Asp Val Gln Glu Ala Gly Ser Cys Val Gln Asp Gly Gln Arg Tyr
1 5 10 15

Asn Asp Lys Asp Val Trp Lys Pro Glu Pro Cys Arg Ile Cys Val Cys
20 25 30

Asp Thr Gly Thr Val Leu Cys Asp Asp Ile Ile Cys Glu Asp Val Lys
35 40 45

Asp Cys Leu Ser Pro Glu Ile Pro Phe Gly Glu Cys Cys Pro Ile Cys
50 55 60

Pro Thr Asp Leu Ala Thr Ala Ser Gly Gln Pro Gly Pro Lys Gly Gln
65 70 75 80

Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile Val Gly Pro Lys Gly Pro
85 90 95

Pro Gly Pro Gln Gly Pro Ala Gly Glu Gln Gly Pro Arg Gly Asp Arg
100 105 110

Gly Asp Lys Gly Glu Lys Gly Ala Pro Gly Pro Arg Gly Arg Asp Gly
115 120 125

Glu Pro Gly Thr Pro Gly Asn Pro Gly Pro Pro Gly Pro Pro Gly Pro
130 135 140

Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln Met Ala Gly
145 150 155 160

Gly Phe Asp Glu Lys Ala Gly Gly Ala Gln Leu Gly Val Met Gln Gly
165 170 175

Pro Met

<210> 7

SEQ listing 32350.ST25

<211> 17
 <212> PRT
 <213> Homo sapiens

<220>
 <221> MOD_RES
 <222> (9)..(9)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, glucose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, glucose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<400> 7

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
 1 5 10 15

Asp

<210> 8
 <211> 19
 <212> PRT
 <213> Homo sapiens

<220>
 <221> MOD_RES
 <222> (9)..(9)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, glucose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, glucose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<400> 8

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
 1 5 10 15

SEQ listing 32350.ST25

Asp Ile Lys

<210> 9
 <211> 20
 <212> PRT
 <213> Homo sapiens

<220>
 <221> MOD_RES
 <222> (9)..(9)
 <223> lysine or hydroxylysine or allysine, may be glycosylated with galactose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (9)..(9)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> lysine or hydroxylysine or allysine, may be glycosylated with galactose, or galactose and glucose

<400> 9

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
 1 5 10 15

Asp Ile Lys Asp
20

<210> 10
 <211> 30
 <212> PRT
 <213> Homo sapiens

<220>
 <221> MOD_RES
 <222> (9)..(9)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<400> 10

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
 1 5 10 15

Asp Ile Lys Asp Ile Val Gly Pro Lys Gly Pro Pro Gly Pro
 Page 15

20 SEQ listing 32350.ST25
25 30

<210> 11
<211> 35
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (9)..(9)
<223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<220>
<221> MOD_RES
<222> (12)..(12)
<223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<400> 11

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
1 5 10 15

Asp Ile Lys Asp Ile Val Gly Pro Lys Gly Pro Pro Gly Pro Gln Gly
20 25 30

Pro Ala Gly
35

<210> 12
<211> 41
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (9)..(9)
<223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<220>
<221> MOD_RES
<222> (12)..(12)
<223> lysine or hydroxylysine or allysine or hydroxyallysine, may be glycosylated with galactose, or galactose and glucose

<400> 12

Gln Asp Val Arg Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly
1 5 10 15

Asp Ile Lys Asp Ile Val Gly Pro Lys Gly Pro Pro Gly Pro Gln Gly
20 25 30

SEQ listing 32350.ST25

Pro Ala Gly Glu Gln Gly Pro Arg Gly
 35 40

<210> 13
 <211> 15
 <212> PRT
 <213> Artificial

<220>
 <223> Homologous region between human, rat and dog collagen propeptide
 <400> 13

Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile
 1 5 10 15

<210> 14
 <211> 1419
 <212> PRT
 <213> Rattus norvegicus
 <400> 14

Met Ile Arg Leu Gly Ala Pro Gln Ser Leu Val Leu Leu Thr Leu Leu
 1 5 10 15

Ile Ala Thr Val Leu Gln Cys Gln Gly Gln Asp Ala Arg Lys Leu Gly
 20 25 30

Pro Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile Ile Gly
 35 40 45

Pro Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala Gly Glu Gln Gly Pro
 50 55 60

Arg Gly Asp Arg Gly Asp Lys Gly Glu Arg Gly Ala Pro Gly Pro Arg
 65 70 75 80

Gly Arg Asp Gly Glu Pro Gly Thr Pro Gly Asn Pro Gly Pro Pro Gly
 85 90 95

Pro Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Gly Asn Phe Ala
 100 105 110

Ala Gln Met Ala Gly Gly Phe Asp Glu Lys Ala Gly Gly Ala Gln Met
 115 120 125

Gly Val Met Gln Gly Pro Met Gly Pro Met Gly Pro Arg Gly Pro Pro
 130 135 140

Gly Pro Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Asn Pro Gly
 145 150 155 160

SEQ listing 32350.ST25

Glu Pro Gly Glu Pro Gly Val Ser Gly Pro Ile Gly Pro Arg Gly Pro
 165 170 175
 Pro Gly Pro Ala Gly Lys Pro Gly Asp Asp Gly Glu Ala Gly Lys Pro
 180 185 190
 Gly Lys Ala Gly Glu Arg Gly Leu Pro Gly Pro Gln Gly Ala Arg Gly
 195 200 205
 Phe Pro Gly Thr Pro Gly Leu Pro Gly Val Lys Gly His Arg Gly Tyr
 210 215 220
 Pro Gly Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala Pro Gly Val Lys
 225 230 235 240
 Gly Glu Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro Gly Pro Met Gly
 245 250 255
 Pro Arg Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly Pro Ala Gly Ala
 260 265 270
 Ala Gly Ala Arg Gly Asn Asp Gly Gln Pro Gly Pro Ala Gly Pro Pro
 275 280 285
 Gly Pro Val Gly Pro Ala Gly Gly Pro Gly Phe Leu Gly Ala Pro Gly
 290 295 300
 Ala Lys Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Ala
 305 310 315 320
 Gln Gly Ser Arg Gly Glu Pro Gly Asn Pro Gly Ser Pro Gly Pro Ala
 325 330 335
 Gly Ala Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro Gly Ala Lys Gly
 340 345 350
 Ser Ala Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Pro
 355 360 365
 Arg Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys
 370 375 380
 Gly Gln Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly
 385 390 395 400
 Pro Lys Gly Glu Thr Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro
 Page 18

Page 19

SEQ Listing 32350.ST25

Pro Gly Thr Asp Gly Pro Lys Gly Ala Ala Gly Pro Asp Gly Pro Pro
660 665 670

Gly Ala Gln Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly
675 680 685

Ala Ala Gly Ile Ala Gly Pro Lys Gly Asp Arg Gly Asp Val Gly Glu
690 695 700

Lys Gly Pro Glu Gly Ala Pro Gly Lys Asp Gly Gly Arg Gly Leu Thr
705 710 715 720

Gly Pro Ile Gly Pro Pro Gly Pro Ala Gly Ala Asn Gly Glu Lys Gly
725 730 735

Glu Val Gly Pro Pro Gly Pro Ser Gly Ser Thr Gly Ala Arg Gly Ala
740 745 750

Pro Gly Glu Arg Gly Glu Thr Gly Pro Pro Gly Pro Ala Gly Phe Ala
755 760 765

Gly Pro Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Asp Gln Gly
770 775 780

Glu Ala Gly Gln Lys Gly Asp Ala Gly Ala Pro Gly Pro Gln Gly Pro
785 790 795 800

Ser Gly Ala Pro Gly Pro Gln Gly Pro Thr Gly Val Thr Gly Pro Lys
805 810 815

Gly Ala Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly
820 825 830

Ala Ala Gly Arg Val Gly Pro Pro Gly Ser Asn Gly Asn Pro Gly Pro
835 840 845

Ala Gly Pro Pro Gly Pro Ala Gly Lys Asp Gly Pro Lys Gly Ala Arg
850 855 860

Gly Asp Thr Gly Ala Pro Gly Arg Ala Gly Asp Pro Gly Leu Gln Gly
865 870 875 880

Pro Ala Gly Ala Pro Gly Glu Lys Gly Glu Pro Gly Asp Asp Gly Pro
885 890 895

Ser Gly Ser Asp Gly Pro Pro Gly Pro Gln Gly Leu Ala Gly Gln Arg
900 905 910

SEQ listing 32350.ST25

Gly Ile Val Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly
 915 920 925
 Leu Pro Gly Pro Ser Gly Glu Pro Gly Lys Gln Gly Ala Pro Gly Ala
 930 935 940
 Ser Gly Asp Arg Gly Pro Pro Gly Pro Val Gly Pro Pro Gly Leu Thr
 945 950 955 960
 Gly Pro Ala Gly Glu Pro Gly Arg Glu Gly Ser Pro Gly Ala Asp Gly
 965 970 975
 Pro Pro Gly Arg Asp Gly Ala Ala Gly Val Lys Gly Asp Arg Gly Glu
 980 985 990
 Thr Gly Ala Leu Gly Ala Pro Gly Ala Pro Gly Pro Pro Gly Ser Pro
 995 1000 1005
 Gly Pro Ala Gly Pro Thr Gly Lys Gln Gly Asp Arg Gly Glu Ala
 1010 1015 1020
 Gly Ala Gln Gly Pro Met Gly Pro Ser Gly Pro Ala Gly Ala Arg
 1025 1030 1035
 Gly Ile Ala Gly Pro Gln Gly Pro Arg Gly Asp Lys Gly Glu Ala
 1040 1045 1050
 Gly Glu Pro Gly Glu Arg Gly Leu Lys Gly His Arg Gly Phe Thr
 1055 1060 1065
 Gly Leu Gln Gly Leu Pro Gly Pro Pro Gly Pro Ser Gly Asp Gln
 1070 1075 1080
 Gly Thr Ser Gly Pro Ala Gly Pro Ser Gly Pro Arg Gly Pro Pro
 1085 1090 1095
 Gly Pro Val Gly Pro Ser Gly Lys Asp Gly Ser Asn Gly Ile Pro
 1100 1105 1110
 Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly Arg Ser Gly Glu Thr
 1115 1120 1125
 Gly Pro Ala Gly Pro Pro Gly Asn Pro Gly Pro Pro Gly Pro Pro
 1130 1135 1140
 Gly Pro Pro Gly Pro Gly Ile Asp Met Ser Ala Phe Ala Gly Leu
 1145 1150 1155

SEQ listing 32350.ST25

Gly Gln Arg Glu Lys Gly Pro Asp Pro Leu Gln Tyr Met Arg Ala
 1160 1165 1170
 Asp Glu Ala Asp Ser Thr Leu Arg Gln His Asp Val Glu Val Asp
 1175 1180 1185
 Ala Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Ser Ile Arg Ser
 1190 1195 1200
 Pro Asp Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys Gln Asp Leu
 1205 1210 1215
 Lys Leu Cys His Pro Glu Trp Lys Ser Gly Asp Tyr Trp Ile Asp
 1220 1225 1230
 Pro Asn Gln Gly Cys Thr Leu Asp Ala Met Lys Val Phe Cys Asn
 1235 1240 1245
 Met Glu Thr Gly Glu Ser Cys Val Tyr Pro Asn Pro Ala Thr Val
 1250 1255 1260
 Pro Arg Lys Asn Trp Trp Ser Ser Lys Ser Lys Glu Lys Lys His
 1265 1270 1275
 Ile Trp Phe Gly Glu Thr Met Asn Gly Gly Phe His Phe Ser Tyr
 1280 1285 1290
 Gly Asp Gly Asn Leu Ala Pro Asn Thr Ala Asn Val Gln Met Thr
 1295 1300 1305
 Phe Leu Arg Leu Leu Ser Thr Glu Gly Ser Gln Asn Ile Thr Tyr
 1310 1315 1320
 His Cys Lys Asn Ser Ile Ala Tyr Leu Asp Glu Ala Ala Gly Asn
 1325 1330 1335
 Leu Lys Lys Ala Leu Leu Ile Gln Gly Ser Asn Asp Val Glu Met
 1340 1345 1350
 Arg Ala Glu Gly Asn Ser Arg Phe Thr Tyr Thr Ala Leu Lys Asp
 1355 1360 1365
 Gly Cys Thr Lys His Thr Gly Lys Trp Gly Lys Thr Ile Ile Glu
 1370 1375 1380
 Tyr Arg Ser Gln Lys Thr Ser Arg Leu Pro Ile Val Asp Ile Ala
 Page 22

SEQ listing 32350.ST25

1385

1390

1395

Pro Met Asp Ile Gly Gly Pro Asp Gln Glu Phe Gly Val Asp Ile
 1400 1405 1410

Gly Pro Val Cys Phe Leu
 1415

<210> 15
 <211> 1067
 <212> PRT
 <213> Canis familiaris

<400> 15

Ser Ala Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Pro
 1 5 10 15

Arg Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys
 20 25 30

Gly Gln Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly
 35 40 45

Pro Lys Gly Glu Pro Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro
 50 55 60

Ala Gly Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Gly Ala
 65 70 75 80

Gly Pro Val Gly Pro Pro Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly
 85 90 95

Phe Pro Gly Gln Asp Gly Leu Ala Gly Pro Lys Gly Ala Pro Gly Glu
 100 105 110

Arg Gly Pro Ser Gly Leu Ala Gly Pro Lys Gly Ala Asn Gly Asp Pro
 115 120 125

Gly Arg Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly
 130 135 140

Arg Pro Gly Asp Ala Gly Pro Gln Gly Lys Val Gly Pro Ser Gly Ala
 145 150 155 160

Pro Gly Glu Asp Gly Arg Pro Gly Pro Pro Gly Pro Gln Gly Ala Arg
 165 170 175

Gly Gln Pro Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Asn Gly
 Page 23

SEQ listing 32350.ST25															
180					185					190					
Glu	Pro	Gly	Lys	Ala	Gly	Glu	Lys	Gly	Leu	Pro	Gly	Ala	Pro	Gly	Leu
		195					200					205			
Arg	Gly	Leu	Pro	Gly	Lys	Asp	Gly	Glu	Thr	Gly	Ala	Ala	Gly	Pro	Pro
	210					215					220				
Gly	Pro	Ala	Gly	Pro	Ala	Gly	Glu	Arg	Gly	Glu	Gln	Gly	Ala	Pro	Gly
225					230					235					240
Pro	Ser	Gly	Phe	Gln	Gly	Leu	Pro	Gly	Pro	Pro	Gly	Pro	Pro	Gly	Glu
				245					250					255	
Gly	Gly	Lys	Pro	Gly	Asp	Gln	Gly	Val	Pro	Gly	Glu	Ala	Gly	Ala	Pro
			260					265					270		
Gly	Leu	Val	Gly	Pro	Arg	Gly	Glu	Arg	Gly	Phe	Pro	Gly	Glu	Arg	Gly
		275					280					285			
Ser	Pro	Gly	Ala	Gln	Gly	Leu	Gln	Gly	Pro	Arg	Gly	Leu	Pro	Gly	Thr
	290					295					300				
Pro	Gly	Thr	Asp	Gly	Pro	Lys	Gly	Ala	Ser	Gly	Pro	Ala	Gly	Pro	Pro
305					310					315					320
Gly	Ala	Gln	Gly	Pro	Pro	Gly	Leu	Gln	Gly	Met	Pro	Gly	Glu	Arg	Gly
				325					330					335	
Ala	Ala	Gly	Ile	Ala	Gly	Pro	Lys	Gly	Asp	Arg	Gly	Asp	Val	Gly	Glu
			340					345					350		
Lys	Gly	Pro	Glu	Gly	Ala	Pro	Gly	Lys	Asp	Gly	Gly	Arg	Gly	Leu	Thr
		355					360					365			
Gly	Pro	Ile	Gly	Pro	Pro	Gly	Pro	Ala	Gly	Ala	Asn	Gly	Glu	Lys	Gly
	370					375					380				
Glu	Val	Gly	Pro	Pro	Gly	Pro	Ala	Gly	Thr	Ala	Gly	Ala	Arg	Gly	Ala
385					390					395					400
Pro	Gly	Glu	Arg	Gly	Glu	Thr	Gly	Pro	Pro	Gly	Pro	Ala	Gly	Phe	Ala
				405					410					415	
Gly	Pro	Pro	Gly	Ala	Asp	Gly	Gln	Pro	Gly	Ala	Lys	Gly	Glu	Gln	Gly
			420					425					430		

SEQ listing 32350.ST25

Glu Ala Gly Gln Lys Gly Asp Ala Gly Ala Pro Gly Pro Gln Gly Pro
 435 440 445

Ser Gly Ala Pro Gly Pro Gln Gly Pro Thr Gly Val Thr Gly Pro Lys
 450 455 460

Gly Ala Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly
 465 470 475 480

Ala Ala Gly Arg Val Gly Pro Pro Gly Ser Asn Gly Asn Pro Gly Pro
 485 490 495

Pro Gly Pro Pro Gly Pro Ser Gly Lys Asp Gly Pro Lys Gly Ala Arg
 500 505 510

Gly Asp Ser Gly Pro Pro Gly Arg Ala Gly Asp Pro Gly Leu Gln Gly
 515 520 525

Pro Ala Gly Pro Pro Gly Glu Lys Gly Glu Pro Gly Asp Asp Gly Pro
 530 535 540

Ser Gly Pro Asp Gly Pro Pro Gly Pro Gln Gly Leu Ala Gly Gln Arg
 545 550 555 560

Gly Ile Val Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly
 565 570 575

Leu Pro Gly Pro Ser Gly Glu Pro Gly Lys Gln Gly Ala Pro Gly Ala
 580 585 590

Ser Gly Asp Arg Gly Pro Pro Gly Pro Val Gly Pro Pro Gly Leu Thr
 595 600 605

Gly Pro Ser Gly Glu Pro Gly Arg Glu Gly Ser Pro Gly Ala Asp Gly
 610 615 620

Pro Pro Gly Arg Asp Gly Ala Ala Gly Val Lys Gly Asp Arg Gly Glu
 625 630 635 640

Thr Gly Pro Val Gly Ala Pro Gly Ala Pro Gly Ser Pro Gly Ser Pro
 645 650 655

Gly Pro Ala Gly Pro Thr Gly Lys Gln Gly Asp Arg Gly Glu Ala Gly
 660 665 670

Ala Gln Gly Pro Met Gly Pro Ala Gly Pro Ala Gly Ala Arg Gly Ile
 675 680 685

SEQ listing 32350.ST25

Pro Gly Pro Gln Gly Pro Arg Gly Asp Lys Gly Glu Ala Gly Glu Ala
 690 695 700
 Gly Glu Arg Gly Leu Lys Gly His Arg Gly Phe Thr Gly Leu Gln Gly
 705 710 715 720
 Leu Pro Gly Pro Pro Gly Pro Ser Gly Asp Gln Gly Ala Ser Gly Pro
 725 730 735
 Ala Gly Pro Ser Gly Pro Arg Gly Pro Pro Gly Pro Val Gly Pro Ser
 740 745 750
 Gly Lys Asp Gly Ala Asn Gly Ile Pro Gly Pro Ile Gly Pro Pro Gly
 755 760 765
 Pro Arg Gly Arg Ser Gly Glu Thr Gly Pro Ala Gly Pro Pro Gly Asn
 770 775 780
 Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Gly Ile Asp Met
 785 790 795 800
 Ser Ala Phe Ala Gly Leu Gly Gln Arg Glu Lys Gly Pro Asp Pro Leu
 805 810 815
 Gln Tyr Met Arg Ala Asp Gln Ala Ala Gly Asp Leu Arg Gln His Asp
 820 825 830
 Ala Glu Val Asp Ala Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Ser
 835 840 845
 Ile Arg Ser Pro Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys Arg
 850 855 860
 Asp Leu Lys Leu Cys His Pro Glu Trp Lys Ser Gly Asp Tyr Trp Ile
 865 870 875 880
 Asp Pro Asn Gln Gly Cys Thr Leu Asp Ala Met Lys Val Phe Cys Asn
 885 890 895
 Met Glu Thr Gly Glu Thr Cys Val Tyr Pro Asn Pro Ala Ser Val Pro
 900 905 910
 Lys Lys Asn Trp Trp Ser Ser Lys Ser Lys Asp Lys Lys His Ile Trp
 915 920 925
 Phe Gly Glu Thr Ile Asn Gly Gly Phe His Phe Ser Tyr Gly Asp Asp
 930 935 940

SEQ listing 32350.ST25

Asn Leu Ala Pro Asn Thr Ala Asn Val Gln Met Thr Phe Leu Arg Leu
 945 950 955 960
 Leu Ser Thr Glu Gly Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser
 965 970 975
 Ile Ala Tyr Leu Asp Glu Ala Ala Gly Asn Leu Lys Lys Ala Leu Leu
 980 985 990
 Ile Gln Gly Ser Asn Asp Val Glu Ile Arg Ala Glu Gly Asn Ser Arg
 995 1000 1005
 Phe Thr Tyr Thr Val Leu Lys Asp Gly Cys Thr Lys His Thr Gly
 1010 1015 1020
 Lys Trp Gly Lys Thr Met Ile Glu Tyr Arg Ser Gln Lys Thr Ser
 1025 1030 1035
 Arg Leu Pro Ile Ile Asp Ile Ala Pro Met Asp Ile Gly Gly Pro
 1040 1045 1050
 Glu Gln Glu Phe Gly Val Asp Ile Gly Pro Val Cys Phe Leu
 1055 1060 1065
 <210> 16
 <211> 50
 <212> PRT
 <213> Canis familiaris
 <400> 16
 Ala Ala Val Leu Arg Cys His Gly Gln Asp Val Arg Gln Pro Gly Pro
 1 5 10 15
 Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile Val Gly Pro
 20 25 30
 Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala Gly Glu Gln Gly Pro Arg
 35 40 45
 Gly Asp
 50

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2005/001152

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/68 A61K31/00 C12Q1/37

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N A61K C12Q

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, COMPENDEX, INSPEC, PAJ, WPI Data, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 780 240 A (SANDELL ET AL) 14 July 1998 (1998-07-14) cited in the application the whole document in particular: column 3, line 35 - column 4, line 30 examples 2,5 claims 1,4	1,3-8
Y		9-12
A		2-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 August 2005

Date of mailing of the international search report

05/09/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Tuynman, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB2005/001152

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MATYAS J R ET AL: "Major role of collagen IIB in the elevation of total type II procollagen messenger RNA in the hypertrophic phase of experimental osteoarthritis." ARTHRITIS AND RHEUMATISM. JUN 1997, vol. 40, no. 6, June 1997 (1997-06), pages 1046-1049, XP008051342 ISSN: 0004-3591 the whole document in particular: abstract page 1047, right-hand column, line 3 - line 20 page 1048, right-hand column, line 38 - line 50	1
Y		9-12
A		2-12
X	----- WO 98/35235 A (UNIVERSITY OF SHEFFIELD; HOLLANDER, ANTHONY, PETER; CROUCHER, LISA, JA) 13 August 1998 (1998-08-13) the whole document in particular: figure 2 page 4, line 21 - line 26 page 5, paragraph 2 - paragraph 3 page 10, line 25 - page 11, line 1	13,14
X	----- WO 02/064625 A (ADP PHARMACEUTICAL PTY LIMITED; THE UNIVERSITY OF SYDNEY; ROUBIN, REBE) 22 August 2002 (2002-08-22) in particular: the whole document sequence 5	13
Y	----- WO 03/021226 A (PHARMACIA CORPORATION; WELSCH, DEAN, J; DUFFIN, KEVIN, L; NEMIROVSKIY,) 13 March 2003 (2003-03-13) cited in the application the whole document in particular: claims 81,83,87	9,10
Y	----- US 2003/219843 A1 (WELSCH DEAN J ET AL) 27 November 2003 (2003-11-27) claim 1 paragraph '0109! paragraph '0160!	11,12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2005/001152

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9-12, 14 (in part)
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 9-12 and 14 are directed to subject matter involving a method of treatment/diagnosis of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB2005/001152

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5780240	A	14-07-1998	US 5541066 A	30-07-1996
WO 9835235	A	13-08-1998	AU 5956098 A	26-08-1998
			EP 0960339 A1	01-12-1999
			WO 9835235 A1	13-08-1998
			JP 2001511253 T	07-08-2001
WO 02064625	A	22-08-2002	WO 02064625 A1	22-08-2002
			CA 2440743 A1	22-08-2002
			EP 1370579 A1	17-12-2003
			JP 2004537274 T	16-12-2004
			NO 20033624 A	10-10-2003
			US 2004157329 A1	12-08-2004
WO 03021226	A	13-03-2003	EP 1497646 A2	19-01-2005
			JP 2005507877 T	24-03-2005
			WO 03021226 A2	13-03-2003
			US 2003054426 A1	20-03-2003
			US 2003049715 A1	13-03-2003
US 2003219843	A1	27-11-2003	NONE	