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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 October 2008 (09.10.2008)

PCT

(10) International Publication Number
WO 2008/119170 A1

(51) International Patent Classification:

G01N 33/53 (2006.01)	*G01N 33/543* (2006.01)
A61K 33/04 (2006.01)	*G01N 33/68* (2006.01)
A61K 45/00 (2006.01)	*C07K 14/52* (2006.01)
A61P 19/08 (2006.01)	*C07K 14/705* (2006.01)
C12Q 1/68 (2006.01)	*C12Q 1/00* (2006.01)

(21) International Application Number:
PCT/CA2008/000595

(22) International Filing Date: 31 March 2008 (31.03.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/909,408	30 March 2007 (30.03.2007)	US
61/025,571	1 February 2008 (01.02.2008)	US

(71) Applicant *(for all designated States except US)*: **CHU SAINTE JUSTINE** [CA/CA]; 3175 Côte-Ste-Catherine, Montréal, Québec, H3T 1C5 (CA).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **MOREAU, Alain** [CA/CA]; 3489 Jules-Huot, Montréal, Québec, H1A 5T5 (CA).

(74) Agent: **DUBUC, J.**; Goudreau Gage Dubuc, 2000 McGill College Avenue, Suite 2200, Montréal, Québec H3A 3H3 (CA).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

WO 2008/119170 A1

(54) Title: METHOD OF DETERMINING RISK OF SCOLIOSIS

(57) Abstract: A method for determining the risk for developing a scoliosis comprising monitoring osteopontin (OPN) expression in a sample from a subject over time; wherein an OPN expression that increases in the subject sample over time is indicative that the subject is at risk for developing a scoliosis.

METHOD OF DETERMINING RISK OF SCOLIOSIS

TITLE OF THE INVENTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority, under 35 U.S.C. § 119(e), of U.S. provisional application serial No. 60/909,408, filed on March 30, 2007 and on U.S. provisional application serial No. 61/025,571, filed on February 1, 2008. All documents above are incorporated herein in their entirety by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] N/A.

FIELD OF THE INVENTION

[0003] The present invention relates to methods of determining the risk of developing scoliosis, methods of stratifying a subject having a scoliosis, methods for assessing the efficacy of a brace on a subject having a scoliosis, and kits therefor.

BACKGROUND OF THE INVENTION

[0004] Spinal deformities and scoliosis in particular, represent the most prevalent type of orthopedic deformities in children and adolescents, while adolescent idiopathic scoliosis (AIS) represents the most common form of scoliosis.

[0005] The etiology of adolescent idiopathic scoliosis (AIS) remains poorly understood resulting in the traditional paradigm that AIS is a multi-factorial disease with a genetic predisposition.[1-7] The occurrence of a melatonin signaling dysfunction in cells derived from biopsies obtained intraoperatively from affected AIS patients has been reported.[8]

[0006] Unfortunately, there is no proven method or test available to identify children or adolescents at risk of developing AIS or to identify, which of the affected individuals may require treatment due to the risk of progression. Consequently, the application of current

treatments, such as bracing or surgical correction, is delayed until a significant deformity is detected or until a significant progression is clearly demonstrated, resulting in a delayed and less optimal treatment.[29]

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

SUMMARY OF THE INVENTION

[0008] More specifically, in accordance with the present invention, there is provided a method for determining the risk for developing a scoliosis comprising monitoring osteopontin (OPN) expression in a sample from a subject over time; wherein an OPN expression that increases in the subject sample over time is indicative that the subject is at risk for developing a scoliosis.

[0009] In a specific embodiment, the monitoring begins when the subject is about three years old. In another specific embodiment, the monitoring is performed by measuring OPN expression at a frequency of at least about once per month. In another specific embodiment, the monitoring is performed by measuring OPN expression at a frequency of at least about once per six month. In another specific embodiment, the method further comprises measuring sCD44 expression in a sample from the subject. In another specific embodiment, the monitoring OPN expression is performed using an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).

[0010] In accordance with the present invention, there is provided a method for determining the risk for developing a scoliosis comprising measuring osteopontin (OPN) expression in a sample from a subject; wherein an OPN expression that is higher in the subject sample than that in a control sample is indicative that the subject is at risk for developing a scoliosis.

[0011] In another specific embodiment, the subject is a likely candidate for developing a scoliosis. In another specific embodiment, the subject is a likely candidate for developing adolescent idiopathic scoliosis. In another specific embodiment, the subject is pre-diagnosed as having a scoliosis.

[0012] In another specific embodiment, the subject is pre-diagnosed with adolescent idiopathic scoliosis.

[0013] In accordance with another aspect of the present invention, there is provided a method of stratifying a subject having a scoliosis comprising measuring osteopontin (OPN) expression in a sample from the subject; whereby the measuring step enables the stratification of the subject into a scoliosis subgroup.

[0014] In accordance with another aspect of the present invention, there is provided a method for assessing the efficacy of a brace on a subject having a scoliosis comprising measuring osteopontin (OPN) expression in a sample from the subject prior to and at least once after bracing the subject, wherein an increase in the OPN expression after as compared to prior to bracing the subject is indicative that the brace is ineffective.

[0015] In a specific embodiment, the determining the OPN expression after the bracing is performed at least one month after the bracing. In another specific embodiment, the determining the OPN expression after bracing the subject is performed at least 2 months hours after the bracing. In another specific embodiment, the determining the OPN expression after bracing the subject is performed at least three months after the bracing. In another specific embodiment, the determining the OPN expression after bracing the subject is performed at least six months after the bracing.

[0016] In another specific embodiment, the method further comprises measuring soluble CD44 receptor (sCD44) expression in the sample from the subject.

[0017] In another specific embodiment, the sample from the subject is a biological fluid from the subject. In another specific embodiment, the biological fluid is selected from the group consisting of blood, urine, tear and saliva. In another specific embodiment, the biological fluid is plasma.

[0018] In another specific embodiment, the OPN expression is OPN protein. In another specific embodiment, the determining of the OPN expression is performed with an antibody that specifically binds to OPN. In another specific embodiment, the measuring OPN expression is performed using an enzyme-linked immunosorbent assay (ELISA). In another specific embodiment, the sample is a plasma sample and an OPN expression that is higher than 700 nanograms per milliliter of plasma is indicative that the subject is at risk for developing a scoliosis. In another specific embodiment, the sample is a plasma sample and an OPN expression that is higher than 800 nanograms per milliliter of plasma is indicative that the subject is at risk for developing a scoliosis.

[0019] In another specific embodiment, the OPN expression is OPN RNA. In another specific embodiment, the sample from the subject is a paraspinal muscle biopsy and the OPN expression is OPN RNA.

[0020] In accordance with another aspect of the present invention, there is provided a method of selecting an agent as a potential candidate for the reduction or prevention of scoliosis comprising contacting a candidate agent with a cell expressing osteopontin (OPN), and detecting the expression of OPN, wherein when the expression of OPN is lower in the presence of the candidate agent as compared to in the absence thereof, the candidate agent is selected.

[0021] In accordance with another aspect of the present invention, there is provided a method of selecting an agent as a potential candidate for the reduction or prevention of scoliosis comprising contacting a candidate agent with a cell expressing sCD44, and detecting the expression of sCD44, wherein when the expression of OPN is higher in the presence of the candidate agent as compared to in the absence thereof, the candidate agent is selected.

[0022] In another specific embodiment, the cell is a cell derived from a scoliotic patient.

[0023] In accordance with another aspect of the present invention, there is provided a method of selecting an agent as a potential candidate for the prevention or reduction of scoliosis comprising administering a candidate agent to a scoliosis model animal before scoliosis has developed in the animal, whereby the candidate is selected when the scoliosis is prevented or reduced in the model animal as compared to in a control animal who was not administered the candidate agent.

[0024] In accordance with another aspect of the present invention, there is provided a method of preventing or reducing scoliosis comprising administering to a subject having scoliosis a therapeutically effective amount of an osteopontin inhibitor (OPN) or a selenium rich diet, whereby scoliosis is thereby prevented or treated.

[0025] In accordance with another aspect of the present invention, there is provided a method of preventing or reducing scoliosis comprising administering to a subject having scoliosis a therapeutically effective amount of a CD44 inhibitor, whereby scoliosis is thereby prevented or treated.

[0026] In accordance with another aspect of the present invention, there is provided a method of preventing or reducing scoliosis comprising administering to a subject having scoliosis a therapeutically effective amount of a sCD44 stimulator, whereby scoliosis is thereby prevented or treated.

[0027] In a specific embodiment of the methods of the present invention, the subject is human. In another specific embodiment of the methods of the present invention, the subject is human female. In another specific embodiment of the methods of the present invention, the subject is human male.

[0028] In accordance with another aspect of the present invention, there is provided an osteopontin inhibitor for use in the treatment or prevention of scoliosis.

[0029] In accordance with another aspect of the present invention, there is provided a CD44 inhibitor for use in the treatment or prevention of scoliosis.

[0030] In accordance with another aspect of the present invention, there is provided a sCD44 stimulator for use in the treatment or prevention of scoliosis.

[0031] In accordance with another aspect of the present invention, there is provided a use of an osteopontin inhibitor in the manufacture of a medicament for the prevention or the treatment of scoliosis.

[0032] In accordance with another aspect of the present invention, there is provided a use of an osteopontin inhibitor for the prevention or the treatment of scoliosis.

[0033] In accordance with another aspect of the present invention, there is provided a use of a CD44 inhibitor in the manufacture of a medicament for the prevention or the treatment of scoliosis.

[0034] In accordance with another aspect of the present invention, there is provided a use of a CD44 inhibitor for the prevention or the treatment of scoliosis.

[0035] In accordance with another aspect of the present invention, there is provided a use of a sCD44 stimulator in the manufacture of a medicament for the prevention or the treatment of scoliosis.

[0036] In accordance with another aspect of the present invention, there is provided a

use of a sCD44 stimulator for the prevention or the treatment of scoliosis.

[0037] In a specific embodiment of the uses of the present invention, the scoliosis is adolescent idiopathic scoliosis.

[0038] In accordance with another aspect of the present invention, there is provided a kit for predicting the risk of developing a scoliosis comprising a ligand specific to osteopontin (OPN) and instructions to use the kit for predicting the risk of developing a scoliosis. In a specific embodiment, the kit further comprises a ligand specific to soluble CD44 (sCD44).

[0039] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] In the appended drawings:

[0041] Figure 1 presents OPN detection in pinealectomized chicken and corresponding scoliosis. Upper and lower panels illustrates the up regulation of OPN expression detected in paraspinal muscles of pinealectomized chicken developing a scoliosis (S) vs. those remaining unaffected (NS) at the mRNA and protein levels respectively.;

[0042] Figure 2 graphically presents in the left panel the dynamic variation of circulating OPN levels in scoliotic bipedal C57Bl/6j mice after surgery, and in the right panel presents typical x-rays of scoliotic deformities observed in bipedal C57Bl/6j mice, where females (708) are more severely affected than males (907);

[0043] Figure 3 shows a variation in plasma melatonin concentrations in different mouse strains. S = scoliotic; NS = non-scoliotic;

[0044] Figure 4 shows the effect of the pharmacological inhibition of OPN transcription on scoliotic pinealectomized chicken;

[0045] Figure 5 graphically presents the sensitivity and specificity of plasma

osteopontin in healthy control subjects, AIS patients and at risk asymptomatic subjects. In Panel A, an analysis that included 33 healthy control subjects and 32 AIS patients with severe Cobb's Angle (≥45°) revealed an area under the curve (AUC) of 0.94 with a standard error of 0.03 (95 percent confidence interval [CI], 0.88 to 1.000). In Panel B, the use of a cut-off value of 700 nanograms per ml of osteopontin showed a high sensitivity (90.6%) and a very good specificity (81.8%) for the early detection of AIS and for detecting the risk of scoliosis progression. In Panel C, the use of a cut-off value of 800 nanograms/ml of osteopontin also showed a high sensitivity (84.9%) and a higher specificity (90.9%) for the early detection of AIS and for detecting the risk of scoliosis progression. In Panel D, a clear correlation between the levels of plasma osteopontin and the Cobb's angle is demonstrated using all AIS patients, yielding a p-value <0.001 and r^2=0.26;

[0046] Figure 6 presents graphs showing the distribution of age in the different groups for male and female combined (control, at risk, AIS <45 and AIS ≥45) (Panel A), and separated by sex female (Panel B) and male (Panel C);

[0047] Figure 7 shows profiles of change in OPN levels, sCD44 levels, and Cobb's angle over follow up time in 4 selected AIS female patients (not under brace treatment) aged 12 (red), 14 (green and blue), and 17 (yellow) at baseline visit;

[0048] Figure 8 shows the distribution of total change in OPN (left panel) and sCD44 (left panel) levels over follow-up time in AIS patients with worsened curve deformity (total increase in Cobb's angle greater than 3°; n=14) and in those without significant change in curve (no change in Cobb's angle, decrease, or increase smaller than 3°; n=36);

[0049] Figure 9 presents graphs showing OPN progression correlated with Cobb's angle progression in AIS patients;

[0050] Figure 10 presents graphs showing OPN regression or stabilization correlated with Cobb's angle regression or stabilization in AIS patients;

[0051] Figure 11 shows profiles of change in OPN and sCD44 levels over follow up time in 4 selected at risk subjects without scoliosis: one male aged 13 (green), and 3 female aged 5 (gold), 11 (blue), and 9 (red) at baseline visit;

[0052] Figure 12 compares OPN, sCD44 and HA levels in non AIS scoliotic patients (NAIS) (OPN (n=28), sCD44 (n=18), HA (n=24)), healthy controls (n=35) and AIS patients (n=252);

[0053] Figure 13 presents a histogram comparison of circulating levels of OPN change in function of spine biomechanics in pre-operated AIS patients (n=79) vs. post-operated AIS patients (n=28);

[0054] Figure 14 presents a histogram comparison of circulating levels of OPN and sCD44 of in pre-operated AIS female (OPN (n=10); sCD44 (n=15)) vs. post-operated AIS female (OPN (n=10); sCD44 (n=12));

[0055] Figure 15 presents charts distributing AIS patients across the predefined cut-off zones pre-operation (Panel A) and post-operation (Panel B);

[0056] Figure 16 presents charts distributing AIS patients across the predefined cut-off zones prior to being treated with bracing (Panel A) and after bracing (Panel B);

[0057] Figure 17 illustrates a hypothetic molecular concept underlying spinal deformity progression in AIS;

[0058] Figure 18 presents a graph that correlates selenium levels in AIS patients with OPN levels;

[0059] Figure 19 presents a histogram comparing selenium levels in three categories of subjects : controls, low OPN producers and high OPN producers;

[0060] Figure 20 presents the nucleotide sequences of the three human OPN isoforms (transcript variant 1, mRNA NM_001040058 (SEQ ID NO: 1); transcript variant 2, mRNA NM_000582 (SEQ ID NO: 2); transcript variant 3, mRNA NM_001040060 (SEQ ID NO: 3) and the amino acid sequences of the three human OPN isoforms (isoform a NP_001035147 (SEQ ID NO: 4); isoform b NP_000573 (SEQ ID NO: 5); and isoform c NP_001035149 (SEQ ID NO: 6));

[0061] Figure 21 presents the nucleotide sequences (mRNA) of six isoforms of human CD44 (NM_000610 transcript variant 1 (SEQ ID NO : 7); NM_001001389 transcript variant 2 (SEQ ID NO: 8); NM_001001390 transcript variant 3 (SEQ ID NO: 9); NM_001001391 transcript variant 4 (SEQ ID NO: 10); NM_001001392 transcript variant

5 (SEQ ID NO: 11); X62739 Isoform identified in tumour cells (SEQ ID NO: 12)) and amino acid sequences of six isoforms of human sCD44 (NP_000601 isoform 1 precursor (SEQ ID NO: 13); NP_001001389 isoform 2 precursor (SEQ ID NO: 14); NP_001001390 isoform 3 precursor (SEQ ID NO: 15); NP_001001391 isoform 4 precursor (SEQ ID NO: 16); NP_001001392 isoform 5 precursor (SEQ ID NO: 17); and CAA44602 Isoform identified in tumour cells (SEQ ID NO: 18)); and

[0062] Figure 22 shows the structure of sCD44 (Panel A), the origin of the various CD44 isoforms (Panel B) and the cleavage site in one sCD44 isoform (SEQ ID NO: 23).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0063] The involvement of osteopontin (OPN) (also called secreted phosphoprotein 1, bone sialoprotein I, early T-lymphocyte activation 1), a multifunctional cytokine, was investigated in adolescent idiopathic scoliosis (AIS) and plasma OPN concentrations were determined in three populations: patients with AIS, healthy controls without any family antecedent for scoliosis and asymptomatic offspring, born from at least one scoliotic parent, who are considered as at risk ("children at risk").

[0064] A group of 252 consecutive patients with AIS were compared with 35 healthy control subjects without any family history of scoliosis and 70 asymptomatic at risk subjects. All subjects were Caucasians and demographic characteristics are shown in Table 2 below. Plasma OPN, soluble CD44 receptor (sCD44), and hyaluronan (HA) levels were measured by enzyme-linked immunosorbent assays. Pinealectomized chicken and genetically modified bipedal C57Bl/6j mice devoid of either OPN or CD44 receptor, a known OPN receptor, were also studied.

[0065] Mean plasma OPN concentration in patients with AIS were significantly higher (p-value <0.001) in patients with AIS having a Cobb's angle >45° (965 ± 414 nanograms per milliliter) than that in healthy controls (570 ± 156 nanograms per milliliter) and than that in AIS patients with a Cobb's angle <45° (799 ± 284 nanograms per milliliter). Diagnostic sensitivity and specificity of OPN for AIS was 84.4 percent and 90.6 percent respectively (cut-off value ≥ 800 nanograms per milliliter). Subgroup analysis showed that 47.9 percent of children at risk had OPN values higher than 800 nanograms per milliliter as opposed to only 8.6 percent for the controls indicating that elevated plasma OPN levels precede scoliosis formation. There were no significant differences in mean plasma sCD44 levels and HA levels between all groups. In respect

to pathophysiology of scoliosis, the bipedal C57Bl/6j mouse model demonstrated that the development of scoliosis requires OPN interactions with CD44 receptors since none of the genetically modified bipedal mice developed a scoliosis. Cut-off values for OPN disclosed herein were calculated using the commercial Elisa kit specific to human OPN from IBL. They may vary when a OPN expression (mRNA or protein) is measured differently (e.g. measuring OPN expression in a different biological sample through OPN RNA or OPN protein but using a different antibody).

[0066] **OPN** (also called secreted phosphoprotein-1, minopontin, or Eta-1) is a phosphorylated glycoprotein containing an arginine-glycine-aspartate (RGD) sequence present in mineralized tissues such as extracellular matrices. This multifunctional cytokine is involved in many pathological conditions.[9,10] The presence of OPN transcripts and proteins in postural control centers such as the cerebellum, skeletal muscle proprioceptive sensory organs, and inner ear structures that control of equilibrium[(11)] is of interest, since AIS patients also exhibit defects in postural control, proprioception and equilibrium.[(12;13)] High plasma OPN levels have been found in different adult cancers and inflammatory conditions[30-33].

[0067] **OPN signaling action:** The OPN signaling pathways are not well understood, although it is known that aside from interacting with integrins, OPN can interact with CD44 receptor at the cell surface.[14,15] Although CD44 is a major receptor for hyaluronan (HA), it also acts as a receptor for OPN and has multiple RGD binding sites. All human isoforms of the CD44 family of adhesion molecules are encoded by a single gene. Alternate splicing of 12 of the 19 exons in the human CD44 gene leads to the production of multiple variant isoforms[16,17] and such structural heterogeneity is responsible of the ligand repertoire of CD44, which includes fibronectin[18], chondroitin sulphate[19], osteopontin[20], at least two heparin binding growth hormones and hyaluronan.[21,22] Soluble variant isoforms of sCD44 (sCD44var) have been associated with several pathological conditions.[16,18,23,24] It has been proposed that sCD44 isoforms are either generated through proteolytic cleavage of cell surface CD44 or by de novo synthesis due to alternative splicing. Functional diversity among CD44 molecules, unrelated to variant exon usage, is demonstrated by observations that CD44H, or any particular splice-variant, can be active for hyaluronan (HA) binding when expressed in some cell types but inactive in others. Many CD44 isoforms are tissue specific, but the full range of soluble variant isoform(s) of sCD44 has been

associated with some pathological conditions. Indeed, circulating levels of total sCD44 and specific soluble CD44 isoforms have been shown to correlate with tumor metastasis in some malignancies, including non-Hodgkin's lymphoma and breast, gastric, and colon carcinomas. The level of soluble CD44 is also known to be higher in the body fluids of subjects with particular inflammatory conditions, such as rheumatoid arthritis, pouchitis and colitis, and bronchitis. Hyaluronan (HA), also called hyaluronate or hyaluronic acid, is a mucopolysaccharide widely distributed throughout the body and produced by a variety of cells including fibroblasts and other specialized connective tissue cells.

[0068] As used herein the term "subject" is meant to refer to any mammal including human, mice, rat, dog, cat, pig, monkey, horse, etc. In a particular embodiment, it refers to a human.

[0069] As used herein the term "brace" is meant to include dental and orthopedic brace and "bracing" thus refers to the action of placing the braces on the subject. In a specific embodiment, it is meant to refer to braces for scoliotic subjects.

[0070] As used herein the terminology "spinal disorders and disorders causing scoliosis" refers to disorders that may involve development of a scoliosis. Without so limited, it includes AIS, congenital scoliosis, congenital cyphose scoliosis, neurological scoliosis, dysplasic scoliosis, neurofibromatosis, cerebral palsy, muscular dystrophies, neuromuscular scoliosis, spondylolesthesis and Noonan syndrome. Scoliosis that may be stratified or predicted excludes those caused by an accident and certain congenital malformations.

[0071] As used herein the terms "likely candidate for developing adolescent idiopathic scoliosis" include children of which a least one parent has adolescent idiopathic scoliosis. Among other factors, age (adolescence), gender and heredity (i.e. born from a mother or father having a scoliosis) are factors that are known to contribute to the risk of developing a scoliosis and are used to a certain degree to assess the risk of developing AIS. In certain subjects, scoliosis develops rapidly over a short period of time to the point of requiring a corrective surgery. Current courses of action available

from the moment AIS is diagnosed (when scoliosis is apparent) include observation (when Cobb's angle is around 10-25°), orthopaedic devices (when Cobb's angle is around 25-30°), and surgery (over 45°). The more reliable methods of determining the risk of progression and of monitoring treatment efficiency in accordance of the present invention may assist in 1) selecting an appropriate diet to remove certain food products identified as contributors to scoliosis; 2) selecting the best therapeutic agent; 3) selecting the least invasive preventive action and/or available treatment such as postural exercises, orthopaedic device, and/or less invasive surgeries or surgeries without fusions (a surgery that does not fuse vertebra and preserves column mobility).

[0072] As used herein, the terms "severe AIS" refers to a scoliosis characterized by Cobb's angle of 45° or more.

[0073] As used herein the terms "risk of developing scoliosis" refer to a genetic or metabolic predisposition of a subject to develop a scoliosis (i.e. spinal deformity) and/or to develop a more severe scoliosis at a future time. For instance, an increase of the Cobb's angle of a subject (e.g. from 40° to 50°, or from 18° to 25°) is a "development" of scoliosis.

[0074] As used herein the terminology "biological sample" refers to any solid or liquid sample isolated from a living being. In a particular embodiment, it refers to any solid or liquid sample isolated from a human. Without being so limited it includes a biopsy material, blood, tears (48), saliva, maternal milk, synovial fluid, urine, ear fluid, amniotic fluid and cerebrospinal fluid. In a specific embodiment it refers to a blood sample.

[0075] As used herein the terminology "blood sample" is meant to refer to blood, plasma or serum. In a preferred embodiment, plasma is used. In a more specific embodiment it refers to a plasma sample.

[0076] As used herein the terminology "control sample" is meant to refer to a sample that does not come from a subject known to have scoliosis or known to be a likely candidate for developing a scoliosis. In methods for determining the risk of

developing scoliosis in a subject that is pre-diagnosed with scoliosis, the sample may however also come from the subject under scrutiny at an earlier stage of the disease or disorder.

[0077] As used herein the term "treating" or "treatment" in reference to scoliosis is meant to refer to at least one of a reduction of Cobb's angle in a preexisting spinal deformity, improvement of column mobility, preservation/maintenance of column mobility, improvement of equilibrium and balance in a specific plan; maintenance/preservation of equilibrium and balance in a specific plan; improvement of functionality in a specific plan, preservation/maintenance of functionality in a specific plan, cosmetic improvement, and combination of any of the above.

[0078] As used herein the term "preventing" or "prevention" in reference to scoliosis is meant to refer to a at least one of a reduction in the progression of a Cobb's angle in a patient having a scoliosis or in an asymptomatic patient, a complete prevention of apparition of a spinal deformity, including changes affecting the rib cage and pelvis in 3D, and a combination of any of the above.

[0079] As used herein the term "osteopontin inhibitor" refers to an agent able to reduce or block expression (transcription or translation) of OPN (gene called sspi1), an agent able to reduce or block OPN secretion or an agent able to reduce or block OPN binding to its receptor CD44. Without being so limited, the agent can be natural or synthetic and can be a protein such as but not limited to an antibody that specifically binds to OPN, a peptide, a small molecule, a nucleotide such as but not limited to an antisense or a siRNA specific to OPN.

[0080] As used herein the term "CD44 inhibitor" refers to an agent able to reduce expression (transcription or translation) of CD44, or an agent able to reduce CD44 localization at the cellular membrane. Without being so limited, the agent can be natural or synthetic and can be a protein such as but not limited to an antibody that specifically binds to CD44, a peptide, a small molecule, a nucleotide such as but not limited to an antisense or a siRNA specific to CD44.

[0081] As used herein the term "sCD44 stimulator" refers to an agent able to increase expression (transcription or translation) of sCD44, an agent able to increase sCD44 secretion or an agent able to increase sCD44 affinity toward OPN. Without being so limited, the agent can be a protein, a peptide, a small molecule or a nucleotide.

[0082] The articles "a," "an" and "the" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[0083] The term "including" and "comprising" are used herein to mean, and re used interchangeably with, the phrases "including but not limited to" and "comprising but not limited to".

[0084] The terms "such as" are used herein to mean, and is used interchangeably with, the phrase "such as but not limited to".

[0085] The present invention also relates to methods for the determination of the level of expression (i.e. transcript or translation product) of OPN, HA or sCD44. The present invention therefore encompasses any known method for such determination including Elisa (Enzyme Linked Immunosorbent Assay), RIA (Radioimmunoassay), real time PCR and competitive PCR, Northern blots, nuclease protection, plaque hybridization and slot blots.

[0086] The present invention also concerns isolated nucleic acid molecules including probes and primers to detect OPN, sCD44 or CD44. In specific embodiments, the isolated nucleic acid molecules have no more than 300, or no more than 200, or no more than 100, or no more than 90, or no more than 80, or no more than 70, or no more than 60, or no more than 50, or no more than 40 or no more than 30 nucleotides. In specific embodiments, the isolated nucleic acid molecules have at least 17, or at least 18, or at least 19, or at least 20, or at least 30, or at least 40 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 300 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 200 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 100 nucleotides. In

other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 90 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 80 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 70 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 60 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 50 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 40 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 17 and no more than 40 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 20 and no more than 30 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 17 and no more than 30 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 300 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 200 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 100 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 90 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 80 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 70 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 60 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 50 nucleotides. In other specific embodiments, the isolated nucleic acid molecules have at least 30 and no more than 40 nucleotides. It should be understood that in real-time PCR, primers also constitute probe without the traditional meaning of this term. Primers or probes appropriate to detect OPN sCD44 and CD44 in the methods of the present invention can be designed with known methods using sequences distributed across their respective nucleotide sequence (49).

[0087] Probes of the invention can be utilized with naturally occurring sugar-phosphate backbones as well as modified backbones including phosphorothioates, dithionates, alkyl phosphonates and α-nucleotides and the like. Modified sugar-phosphate backbones are generally known. Probes of the invention can

be constructed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), and preferably of DNA.

[0088] The types of detection methods in which probes can be used include Southern blots (DNA detection), dot or slot blots (DNA, RNA), and Northern blots (RNA detection). Although less preferred, labeled proteins could also be used to detect a particular nucleic acid sequence to which it binds. Other detection methods include kits containing probes on a dipstick setup and the like.

[0089] As used herein the terms "detectably labeled" refer to a marking of a probe or an antibody in accordance with the presence invention that will allow the detection of OPN, HA and/or sCD44 in accordance with the present invention. Although the present invention is not specifically dependent on the use of a label for the detection of a particular nucleic acid sequence, such a label might be beneficial, by increasing the sensitivity of the detection. Furthermore, it enables automation. Probes can be labeled according to numerous well known methods. Non-limiting examples of labels include 3H, 14C, 32P, and 35S. Non-limiting examples of detectable markers include ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radionucleotides. It will become evident to the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe.

[0090] As commonly known, radioactive nucleotides can be incorporated into probes of the invention by several methods. Non-limiting examples thereof include kinasing the 5' ends of the probes using gamma 32P ATP and polynucleotide kinase, using the Klenow fragment of Pol I of E. coli in the presence of radioactive dNTP (e.g. uniformly labeled DNA probe using random oligonucleotide primers in low-melt gels), using the SP6/T7 system to transcribe a DNA segment in the presence of one or more radioactive NTP, and the like.

[0091] The present invention also relates to methods of selecting compounds. As used herein the term "compound" is meant to encompass natural, synthetic or semi-synthetic compounds, including without being so limited chemicals,

macromolecules, cell or tissue extracts (from plants or animals), nucleic acid molecules, peptides, antibodies and proteins.

[0092] The present invention also relates to arrays. As used herein, an "array" is an intentionally created collection of molecules which can be prepared either synthetically or biosynthetically. The molecules in the array can be identical or different from each other. The array can assume a variety of formats, e.g., libraries of soluble molecules; libraries of compounds tethered to resin beads, silica chips, or other solid supports.

[0093] As used herein “array of nucleic acid molecules" is an intentionally created collection of nucleic acids which can be prepared either synthetically or biosynthetically in a variety of different formats (e.g., libraries of soluble molecules; and libraries of oligonucleotides tethered to resin beads, silica chips, or other solid supports). Additionally, the term "array" is meant to include those libraries of nucleic acids which can be prepared by spotting nucleic acids of essentially any length (e.g., from 1 to about 1000 nucleotide monomers in length) onto a substrate. The term "nucleic acid" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides, deoxyribonucleotides or peptide nucleic acids (PNAs), that comprise purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups, as may typically be found in RNA or DNA, or modified or substituted sugar or phosphate groups. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. Thus the terms nucleoside, nucleotide, deoxynucleoside and deoxynucleotide generally include analogs such as those described herein. These analogs are those molecules having some structural features in common with a naturally occurring nucleoside or nucleotide such that when incorporated into a nucleic acid or oligonucleotide sequence, they allow hybridization with a naturally occurring nucleic acid sequence in solution. Typically, these analogs are derived from naturally occurring nucleosides and nucleotides by replacing and/or modifying the base, the ribose or the phosphodiester moiety. The changes can be tailor made to stabilize or destabilize hybrid formation or enhance the specificity of hybridization with a complementary nucleic acid sequence as desired.

[0094] As used herein "solid support", "support", and "substrate" are used interchangeably and refer to a material or group of materials having a rigid or semi-rigid surface or surfaces. In many embodiments, at least one surface of the solid support will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. According to other embodiments, the solid support(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations.

[0095] Any known nucleic acid arrays can be used in accordance with the present invention. For instance, such arrays include those based on short or longer oligonucleotide probes as well as cDNAs or polymerase chain reaction (PCR) products. Other methods include serial analysis of gene expression (SAGE), differential display, as well as subtractive hybridization methods, differential screening (DS), RNA arbitrarily primer (RAP)-PCR, restriction endonucleolytic analysis of differentially expressed sequences (READS), amplified restriction fragment-length polymorphisms (AFLP).

Antibodies

[0096] The present invention encompasses using antibodies for detecting or determining OPN, sCD44 or CD44 levels for instance in the samples of a subject and for including in kits of the present invention. Antibodies that specifically bind to these biological markers can be produced routinely with methods further described below. The present invention also encompasses using antibodies commercially available. Without being so limited antibodies that specifically bind to OPN include those listed in Table 1 below.

[0097] Table 1 commercially available human OPN Elisa kits.

Company	Kit name	Catalogue number	Sensitivity
IBL Hambourg	Human Osteopontin ELISA	JP 171 58	3,33ng/ml
IBL America	Human Osteopontin N-Half Assay Kit - IBL	27258	3,90 pmol/L
IBL-America	Human Osteopontin Assay Kit - IBL	27158	3,33ng/ml
Assay designs	Osteopontin (human) EIA Kit	900-142	0,11ng/ml
American Research Products, Inc.	Osteopontin, human kit	17158	?
R&D Systems	Human Osteopontin (OPN) ELISA Kit	DOST00	0.024 ng/mL
Promokine	Human Osteopontin ELISA	PK-EL-KA4231	3,6ng/ml
.Uscnlife	Human Osteopontin,OPN ELISA Kit	E0899h	?

[0098] Both monoclonal and polyclonal antibodies directed to OPN are included within the scope of this invention as they can be produced by well established procedures known to those of skill in the art. Additionally, any secondary antibodies, either monoclonal or polyclonal, directed to the first antibodies would also be included within the scope of this invention.

[0099] As used herein, the term "anti-OPN antibody" or "immunologically specific anti-OPN antibody" refers to an antibody that specifically binds to (interacts with) an OPN protein and displays no substantial binding to other naturally occurring proteins other than the ones sharing the same antigenic determinants as the OPN protein. The term antibody or immunoglobulin is used in the broadest sense, and covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies, and antibody fragments so long as they exhibit the desired biological activity. Antibody fragments comprise a portion of a full length antibody,

generally an antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, single domain antibodies (*e.g.,* from camelids), shark NAR single domain antibodies, and multispecific antibodies formed from antibody fragments. Antibody fragments can also refer to binding moieties comprising CDRs or antigen binding domains including, but not limited to, VH regions (V_H, V_H-V_H), anticalins, PepBodies™, antibody-T-cell epitope fusions (Troybodies) or Peptibodies. Additionally, any secondary antibodies, either monoclonal or polyclonal, directed to the first antibodies would also be included within the scope of this invention.

[00100] In general, techniques for preparing antibodies (including monoclonal antibodies and hybridomas) and for detecting antigens using antibodies are well known in the art (Campbell, 1984, In "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", Elsevier Science Publisher, Amsterdam, The Netherlands) and in Harlow et al., 1988 (in: Antibody A Laboratory Manual, CSH Laboratories). The term antibody encompasses herein polyclonal, monoclonal antibodies and antibody variants such as single-chain antibodies, humanized antibodies, chimeric antibodies and immunologically active fragments of antibodies (e.g. Fab and Fab' fragments) which inhibit or neutralize their respective interaction domains in Hyphen and/or are specific thereto.

[00101] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc), intravenous (iv) or intraperitoneal (ip) injections of the relevant antigen with or without an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.,* keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

[00102] Animals may be immunized against the antigen, immunogenic conjugates, or derivatives by combining the antigen or conjugate (*e.g.,* 100 µg for rabbits or 5 µg for mice) with 3 volumes of Freund's complete adjuvant and injecting the solution

intradermally at multiple sites. One month later the animals are boosted with the antigen or conjugate (*e.g.,* with 1/5 to 1/10 of the original amount used to immunize) in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, for conjugate immunizations, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[00103] Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, Nature, 256: 495 (1975), or may be made by recombinant DNA methods (*e.g.,* U.S. Patent No. 6,204,023). Monoclonal antibodies may also be made using the techniques described in U.S. Patent Nos. 6,025,155 and 6,077,677 as well as U.S. Patent Application Publication Nos. 2002/0160970 and 2003/0083293 (see also, *e.g.*, Lindenbaum et al., 2004).

[00104] In the hybridoma method, a mouse or other appropriate host animal, such as a rat, hamster or monkey, is immunized (*e.g.,* as hereinabove described) to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the antigen used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell.

[00105] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00106] As used herein, the term "purified" in the expression "purified antibody" is simply meant to distinguish man-made antibody from an antibody that may

naturally be produced by an animal against its own antigens. Hence, raw serum and hybridoma culture medium containing anti-OPN antibody are "purified antibodies" within the meaning of the present invention.

[00107] The present invention also encompasses arrays to detect and/or quantify the translation products of OPN, HA or sCD44. Such arrays include protein micro- or macroarrays, gel technologies including high-resolution 2D-gel methodologies, possibly coupled with mass spectrometry imaging system at the cellular level such as microscopy combined with a fluorescent labeling system.

[00108] The present invention also encompasses methods for identifying specific mutation(s) directly or indirectly affecting the transcription, translation, post-translational modification or activity of OPN. Without being so limited, mutations of interest include any mutation affecting the interactions between OPN and any soluble or non soluble isoform of CD44 or the binding of HA to any soluble or non soluble isoform of CD44.

[00109] The present invention also encompasses the monitoring of the biomarkers disclosed herein to assess the efficacy of numerous approaches to prevent scoliosis and curve progression such as any physical therapies (e.g. postural exercises, physiotherapies, biomechanical stimulations by manipulation or using specific devices e.g. vibrant plates); the monitoring of bracing efficacy or development of novel braces; the monitoring of new surgical devices with or without fusion of vertebras, and the monitoring of the efficacy of specific diet, nutraceutical and/or pharmacological treatments. Without being so limited, the first measure after the braces have been applied could be performed 1 month later to determine for instance whether the braces are well adjusted and determine whether the patient is compliant to the treatment. Thereafter, the monitoring could be performed every three to six months depending on whether high OPN levels are detected or not. This method of the present invention may advantageously reduces the requirement for x-rays. X-rays could be performed for instance only at visits where OPN levels detected are too high.

[00110] The present invention also encompasses the monitoring of the biomarkers disclosed herein identify patients having a risk of progression for early

bracing or for less-invasive surgeries with novel fusionless devices, for pharmacological treatments and to monitor responses to treatment in patients with AIS. Of note, fusionless devices are particularly useful for patients still possessing a growth potential so that identification of the risk of developing a scoliosis as early as possible in the life of the subject is beneficial. In a specific embodiment, monitoring begins when the subject is about 5 years old or less in subjects having a scoliosis family antecedent/history. The frequency of the testing could typically be every six months. In case where OPN values are above the cut-off value (i.e. > 800 ng/ml when the OPN IBL ELISA kit code No. 27158 is used), the frequency would be advantageously significantly increased (e.g. every month, every two months, every three months...).

[00111] The present invention also encompasses methods to screen/select for potential useful therapeutic agents using whole cells assays, the therapeutic compound being able to repress the transcription and/or synthesis of OPN (encoded by ssp1 gene), and/or able to increase the production of sCD44 which could sequester circulating OPN, and/or able to interfere with OPN liaison with the CD44 receptor, and/or able to block CD44 receptor. Cells for use in such methods includes cells of any source (including in house or commercially available cell lines) and type (any tissue). In house cell lines could be made for instance by immortalizing cells from AIS subjects. In specific embodiments, methods of screening of the invention seek to identify agents that inhibit OPN expression (transcription and/or translation) and agents that increase sCD44 expression (transcription and/or translation). Useful cell lines for these embodiments include those producing high levels of OPN and/or low levels of sCD44. Such useful cell lines are described in references 43-56.

[00112] In a particular embodiment, it includes cells of any cell type derived from a scoliotic patient. (whole cell assay). In specific embodiments, it includes osteoblasts, chondrocytes, myoblasts or blood cells including lymphocytes. As used herein, the term "cell derived from a scoliotic patient" refers to cells isolated directly from scoliotic patients, or immortalized cell lines originating from cells isolated directly from scoliotic patients. In specific embodiments, the cells are paraspinal muscle cells. Such cells may be isolated by a subject through needle biopsies for instance.

[00113] Pharmaceutical compositions can also be administered by routes

such as nasally, intravenously, intramuscularly, subcutaneously, sublingually, intrathecally, or intradermally. The route of administration can depend on a variety of factors, such as the environment and therapeutic goals.

Dosage

[00114] Any amount of a pharmaceutical and/or nutraceutical and/or dietary supplement compositions can be administered to a subject. The dosages will depend on many factors including the mode of administration. Typically, the amount of anti-scoliosis composition (e.g. osteopontin inhibitor or selenium compound) contained within a single dose will be an amount that effectively prevents, delays or reduces scoliosis without inducing significant toxicity "therapeutically effective amount".

[00115] In some embodiments, the therapeutically effective amount of the neutraceutical anti-scoliosis composition (e.g. selenium supplement) can be altered. Useful effective amount concentrations include amounts ranging from about 0.01% to about 10% of a total diet on a weight by weight basis, from about 1% to about 6% of a total diet on a weight by weight basis, or from about 02% to about 6% of a total diet on a weight by weight basis.

[00116] The effective amount of the osteopontin inhibitor or selenium compound may also be measured directly. The effective amount may be given daily or weekly or fractions thereof. Typically, a pharmaceutical and/or nutraceutical and/or dietary supplement composition of the invention can be administered in an amount from about 0.001 mg up to about 500 mg per kg of body weight per day (e.g., 10 mg, 50 mg, 100 mg, or 250 mg). Dosages may be provided in either a single or multiple dosage regimen. For example, in some embodiments the effective amount is a dose that ranges from about 1 mg to about 25 grams of the anti-scoliose preparation per day, about 50 mg to about 10 grams of the anti-scoliose preparation per day, from about 100 mg to about 5 grams of the anti-scoliose preparation per day, about 1 gram of the anti-scoliose preparation per day, about 1 mg to about 25 grams of the anti-scoliose preparation per week, about 50 mg to about 10 grams of the anti-scoliose preparation per week, about 100 mg to about 5 grams of the anti-scoliose preparation every other day, and about 1 gram of the anti-scoliose preparation once a week.

[00117] By way of example, a pharmaceutical (e.g. containing an osteopontin inhibitor) and/or nutraceutical (e.g. containing selenium) and/or dietary supplement (e.g. containing selenium) composition of the invention can be in the form of a liquid, solution, suspension, pill, capsule, tablet, gelcap, powder, gel, ointment, cream, nebulae, mist, atomized vapor, aerosol, or phytosome. For oral administration, tablets or capsules can be prepared by conventional means with at least one pharmaceutically acceptable excipient such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets can be coated by methods known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups, or suspension, or they can be presented as a dry product for constitution with saline or other suitable liquid vehicle before use. Dietary supplements of the invention also can contain pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles, preservatives, buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration also can be suitably formulated to give controlled release of the active ingredients.

[00118] In addition, a pharmaceutical (e.g. containing an osteopontin inhibitor) and/or nutraceutical (e.g. containing selenium) and/or dietary supplement (e.g. containing selenium) composition of the invention can contain a pharmaceutically acceptable carrier for administration to a mammal, including, without limitation, sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents include, without limitation, propylene glycol, polyethylene glycol, vegetable oils, and injectable organic esters. Aqueous carriers include, without limitation, water, alcohol, saline, and buffered solutions. Pharmaceutically acceptable carriers also can include physiologically acceptable aqueous vehicles (e.g., physiological saline) or other known carriers appropriate to specific routes of administration.

[00119] An osteopontin inhibitor or selenium may be incorporated into dosage forms in conjunction with any of the vehicles which are commonly employed in pharmaceutical preparations, e.g. talc, gum arabic, lactose, starch, magnesium searate, cocoa butter, aqueous or non-aqueous solvents, oils, paraffin derivatives or glycols. Emulsions such as those described in U.S. Pat. No. 5,434,183, may also be used in which vegetable oil (e.g., soybean oil or safflower oil), emulsifying agent (e.g., egg yolk phospholipid) and water are combined with glycerol. Methods for preparing appropriate

formulations are well known in the art (see e.g., Remington's Pharmaceutical Sciences, 16th Ed., 1980, A. Oslo Ed., Easton, Pa.).

[00120] In cases where parenteral administration is elected as the route of administration, preparations containing osteopontin inhibitor or selenium may be provided to patients in combination with pharmaceutically acceptable sterile aqueous or non-aqueous solvents, suspensions or emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil, fish oil, and injectable organic esters. Aqueous carriers include water, water-alcohol solutions, emulsions or suspensions, including saline and buffered medical parenteral vehicles including sodium chloride solution, Ringer's dextrose solution, dextrose plus sodium chloride solution, Ringer's solution containing lactose, or fixed oils. Intravenous vehicles may include fluid and nutrient replenishers, electrolyte replenishers, such as those based upon Ringer's dextrose, and the like.

[00121] These are simply guidelines since the actual dose must be carefully selected and titrated by the attending physician based upon clinical factors unique to each patient or by a nutritionist. The optimal daily dose will be determined by methods known in the art and will be influenced by factors such as the age of the patient and other clinically relevant factors. In addition, patients may be taking medications for other diseases or conditions. The other medications may be continued during the time that the osteopontin inhibitor or selenium compound is given to the patient, but it is particularly advisable in such cases to begin with low doses to determine if adverse side effects are experienced.

[00122] The present invention also relates to kits. Without being so limited, it relates to kits for stratifying scoliotic subjects and/or predicting whether a subject is at risk of developing a scoliosis comprising an isolated nucleic acid, a protein or a ligand such as an antibody in accordance with the present invention as described above. For example, a compartmentalized kit in accordance with the present invention includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or

solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the subject sample (DNA genomic nucleic acid, cell sample or blood samples), a container which contains in some kits of the present invention, the probes used in the methods of the present invention, containers which contain enzymes, containers which contain wash reagents, and containers which contain the reagents used to detect the extension products. Kits of the present invention may also contain instructions to use these probes and or antibodies to stratify scoliotic subjects or predict whether a subject is at risk of developing a scoliosis.

[00123] The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLE 1

Material and methods

[00124] **GENERATION OF BIPEDAL C57BL/6J OPN-NULL AND CD44-NULL MICE.** Experiments in mice were conducted according to protocols approved by The Ste-Justine Hospital's Animal Health Care Review Committee. Breeding pairs of C57Bl/6 devoid of either OPN (OPN-null mice) or CD44 receptor (CD44-null mice) backcrossed for more than 10 generations in C57Bl/6j mice were graciously obtained from Dr. Susan Rittling, (Rutger University, NJ, USA) and Dr. Tak Mak (University of Toronto, ON, Canada), respectively, to establish new colonies, while C57Bl/6j mice served as wild-type control mice (Charles-River, Wilmington, MA, USA). The C57Bl6/6j mouse strain was used because it is naturally deficient in melatonin[26], exhibits high circulating OPN levels[27] and develops scoliosis when they are maintained in a bipedal state. [28] It is a well known scoliosis animal model. Bipedal surgeries were performed after weaning by amputation of the forelimbs and tail under anesthesia as reported previously.[28] All mice underwent complete radiographic examination under anesthesia using a Faxitron™ X-rays apparatus (Faxitron X-rays Corp. Wheeling, IL, USA) every two weeks starting at the age of six weeks. Anteroposterior X-rays were taken and each digital image was evaluated subsequently for the presence of scoliosis. Cobb's angle threshold value of 10° or higher was retained as a significant scoliotic condition.

[00125] **IMMUNODETECTION OF MOUSE OPN** Mouse serum was obtained from

peripheral blood samples for the determination of serum levels of OPN and were collected in serum separator tubes containing silica gel (BD Microtainer, BD New Jersey, USA) and then centrifuged. Derived serum samples were aliquoted and kept frozen at -80°C until thawed and analyzed. Serum concentrations of OPN were measured by capture enzyme-linked immunosorbent assays (ELISA) according to the protocol provided by the manufacturer (IBL, Hamburg, Germany). The OPN ELISA kit measured total concentration of both phosphorylated and non-phosphorylated of all isoforms of OPN in serum. ELISA tests were performed in duplicate and the optical density was measured at 450 nm using an AsysHiTech™ Expert-96 microplate reader (Biochrom, Cambridge, UK). Although serum was used in mice herein, the present invention also encompasses measuring OPN in mice plasma.

[00126] **GENERATION OF PINEALECTOMIZED CHICKENS.** A percentage of pinealectomized chickens develop a scoliosis and they are thus used as a scoliosis model. For this study, 145 newly hatched chickens (Mountain Hubbard) were purchased at a local hatchery and pinealectomy were performed as previously described[25].

[00127] **EXPRESSION ANALYSIS AND IMMUNODETECTION OF CHICKEN OPN.** Total cellular RNA was prepared from paraspinal muscles of pinealectomized chickens by phenol/chloroform extraction. For RT-PCR, 1 microgramme total RNA was reversed transcribed using ThermoScript™ reverse transcriptase (Invitrogen), and the equivalent of 0.1 microgramme of reverse-transcribed RNA used for PCR reactions. These were carried out in a final volume of 50 microliters containing 200 micromolars dNTPs, 1.5 millimolars $MgCl_2$, 10 picomolars each primer, and 1U Pfu DNA-polymerase (Stratagene, LaJolla, CA, USA). PCR reactions were performed using the following primers and conditions: chicken OPN (420 bp PCR product): 5'-ACACTTTCACTCCAATCGTCC -3' (SEQ ID NO: 19)(forward), 5'-TGCCCTTTCCGTTGTTGTCC-3' (SEQ ID NO: 20) (reverse) 35 cycles: 95°C/45 seconds, 66°C/45 seconds, 72°C/1 minute. For quantitative analysis, all amplifications were normalized against that of the housekeeping gene β-actin; chicken β-actin (460 bp PCR product) 5'-GGAAATCGTGCGTGACAT-3' (SEQ ID NO: 21) (forward), 5'-TCATGATGGAGTTGAATGTAGTT-3' (SEQ ID NO: 22) (reverse) 32 cycles: 94°C/ 45 seconds, 55°C/45 seconds, 72°C/1 minute. PCR amplified products were analyzed on 1.5% agarose gel containing ethidium bromide. Total protein extracts of paraspinal muscles were used to detect chicken OPN by Western blot using anti-human OPN

antibodies cross-reacting with chicken OPN (clone 8E5, Kamiya Biomedial , WA, USA).

[00128] **HUMAN POPULATIONS** The institutional review boards of The Sainte-Justine Hospital, The Montreal Children's Hospital, The Shriners Hospital for Children in Montreal, McGill University and The Affluent School Board, approved the study. Parents or legal guardians of all participants gave written informed consent, and minors gave their assent.

[00129] All patients with AIS were examined by one of six orthopedic surgeons. A person was deemed to be affected if history and physical examination were consistent with the diagnosis of idiopathic scoliosis and a minimum of a ten degree curvature in the coronal plane with vertebral rotation was found on a standing radiograph of the spine. Healthy controls were recruited in elementary schools of Montreal. Each subject was examined by the same orthopedic surgeon using Adam's forward bending-test with a scoliometer.

[00130] Three populations were investigated: patients with AIS, healthy controls without any family antecedent/history for scoliosis and asymptomatic offspring, born from at least one scoliotic parent, who are considered as at risk of developing a scoliosis. A group of 252 consecutive patients with AIS, 35 healthy control subjects and 70 asymptomatic children at risk of developing a scoliosis were recruited. All subjects were Caucasians and demographic characteristics are shown in Table 2 below).

Table 2. Demographic and clinical characteristics of patients with AIS, healthy control and at risk control subjects.

Characteristics	Subject Type							
	AIS				Healthy Control Subjects		At Risk Control Subjects	
	Female		Male		Female	Male	Female	Male
Number	215		37		19	16	45	25
Mean Age (Years)	14.1 ± 2.1		14.8 ± 2.2		10.6 ± 0.6	10.9 ± 0.6	9.8 ± 3.7	10.0 ± 2.9
Patient percentage & Mean Cobb's Angle								
Thoracolumbar	*35.8%*	22.5 ± 15.2	*29.7%*	28.3 ± 22.8	-	-	-	-
Thoracic	*20.5%*	39.7 ± 20.4	*29.7%*	34.1 ± 22.3	-	-	-	-
Double Scoliosis (Thoracic + Lumbar)	*30.2%*		*24.3%*		-	-	-	-
Thoracic Curvature		34.8 ± 19.0		38.9 ± 21.2				
Lumbar Curvature		31.0 ± 17.3		33.0 ± 18.7				
Lumbar	*4.7%*	25.4 ± 10.7	*8.1%*	20.3 ± 3.5	-	-	-	-
Double Scoliosis (Thoracic + Thoracolumbar)	*6.0%*		*5.4%*		-	-	-	-
Thoracic Curvature		25.4 ± 13.5		36.0 ± 19.8				
Lumbar Curvature		25.2 ± 15.5		41.0 ± 29.7				
Triple Scoliosis	*1.9%*	36.8 ± 18.5	*2.7%*	8.0	-	-	-	-
		41.0 ± 14.3		11.0				
		30.5 ± 7.7		11.0				
Double Scoliosis (Thoracic+Thoracic)	0.9%		-		-	-	-	-
		29.0 ± 5.7		-				

	16.5 ± 3.5	-				
Heredity	36.3 %	37.8%	0.0%	0.0%	100.0%	100.0%

* Plus–minus values are means ± standard deviations. † Mean Cobb's Angles for double scoliosis are represented by the curvatures on the thoracic and lumbar levels separately.
‡ Mean Cobb's Angle for the triple scoliosis represents two thoracic curvatures and one lumbar curvature.

[00131] **OSTEOPONTIN, sCD44 AND HA ENZYME-LINKED IMMUNOSORBENT ASSAYS** Peripheral blood samples for AIS patients, asymptomatic children and control groups were collected in EDTA-containing tubes and then centrifuged. Derived plasma samples were aliquoted and kept frozen at -80°C until thawed and analyzed. Plasma concentrations of OPN and sCD44 were measured by capture enzyme-linked immunosorbent assays (ELISA) according to protocols provided by the manufacturer (IBL, Hamburg, Germany). The sCD44 Elisa kit (sCD44std) measured all circulating (soluble) CD44 isoforms comprising the standard protein sequences but not the rare isoforms associated with alternative splicing between exons V2 and V10 (50) (see also Figure 22). The OPN IBL ELISA kit (code No. 27158) measures total concentration of both phosphorylated and non-phosphorylated of all isoforms of OPN in plasma. Circulating levels of HA were measured in all plasma samples using an ELISA kit (HA-Elisa (K-1200), Echelon Biosciences, Salt Lake City, UT). All ELISA tests were performed in duplicate and the optical density was measured at 450 nm (for OPN and sCD44) and 405 nm (for HA) using an AsysHiTech Expert-96™ microplate reader (Biochrom, Cambridge, UK). Other Elisa kits available commercially or house made can be used in methods of the present invention. The cut-off value that statistically distinguishes non scoliotic subjects from scoliotic subjects that will help predict the risk of scoliosis progression as determined with these other kits will likely differ from that calculated with the kit used herein. It may however be calculated for each new antibody used as described herein.

[00132] **STATISTICAL ANALYSIS** Age and gender differences among the different AIS and control groups were assessed using Pearson's Chi-square and Student's t tests, respectively. Multiple linear regression models were used to test for association between groups and levels of OPN, sCD44, and HA. Values were adjusted for age, gender, and age-gender interaction when these potential confounders were associated with the biomarker levels at $p<0.1$. Interactions between group and gender were also investigated. It was first tested for an overall group effect using a global F test comparing models with and without group effects. Were then tested specific differences between groups, applying a Bonferroni correction for multiple testing. Receiver-operating characteristics (ROC) curves were used to evaluate the diagnostic value of OPN, and to identify the optimal threshold values. The sensitivity (proportion of true-positive results when the assay was applied to patients known to have AIS) and specificity (proportion of true-negative results when the assay was applied to healthy controls) of OPN were

profiled by curves. The area under ROC curve (AUC) and associated 95% confidence interval were calculated. The test of the hypothesis that the theoretical AUC is 0.5 was based on the confidence interval. Statistical analysis was performed with the SAS software, version 9.1, with the exception of the ROC curve analysis, which was performed with the ROCR package for R (www.r-project.org) [51,52]. In all analyses except when otherwise mentioned a *p*-value < 0.05 was considered statistically significant.

EXAMPLE 2

mRNA and protein OPN levels pinealectomized chicken

[00133] Expression analysis and immunodetection analysis of OPN in pinealectomized chicken were performed as described in Example 1 above. OPN at the mRNA and protein levels occurring in pinealectomized chicken were measured. Figure 1 shows a strong increase of OPN at the mRNA and protein levels only in pinealectomized chicken that developed a scoliosis.

EXAMPLE 3

OPN protein levels in C57Bl/6j mice

[00134] Bipedal C57Bl/6j mice were generated and their OPN level was determined as described in Example 1 above. Bipedal ambulation for 8 weeks in C57Bl/6j mice induced scoliosis at a rate of 46 percent in females and 24 percent in males which correlated well with higher plasma OPN levels found in females (Table 3 below). The relevance of this animal model is strengthened by the fact that scoliosis are more frequently seen in number and severity in bipedal C57Bl/6j females (46%) when compared to bipedal males (24%) as is also observed in humans.

[00135] Table 3. Scoliosis frequency in naturally melatonin deficient mouse strain C57Bl/6j mice and genetically modified C57Bl mice devoid of OPN or CD44.

		n	% of scoliosis	Mean period of follow-up
C57Bl/6j	♂	21	24%	57 weeks +/- 3
	♀	28	46%	57 weeks +/- 3
C57Bl/6j OPN-null	♂	30	0%	54 weeks +/- 2
	♀	24	0%	54 weeks +/- 2
C57Bl/6j CD44-null	♂	29	0%	52 weeks +/- 2
	♀	31	0%	52 weeks +/- 2

[00136] Figure 2 shows that the OPN protein level strongly increases after bipedal surgery (i.e. during scoliosis development) in scoliotic C57Bl/6j mice.

EXAMPLE 4

Observation of effect of absence of OPN or CD44 bipedal C57Bl/6j mice on scoliosis

[00137] The contribution of OPN and CD44 receptor as an integral part of the pathophysiology cascade in scoliosis formation and curve progression was also examined by studying genetically modified bipedal C57Bl/6j mice by conducting experiments as described in Example 1 above. As shown in Table 3 above, it was found that none of the bipedal C57Bl/6j OPN-null (n=54) and C57Bl/6j CD44-null mice (n=60) respectively, developed a scoliosis even if their analysis was extended over 52 weeks. Scoliosis development is detected 8 weeks after the surgery. A longer follow-up was performed to demonstrate that scoliosis development was not simply delayed in OPN-null and CD44-null mice.

[00138] In parallel, melatonin circulating levels were measured in wild-type and OPN-KO mice to exclude the possibility that absence of scoliosis in bipedal C57Bl/6 OPN-KO mice was due to an increased production of melatonin.

[00139] Figure 3 shows a two-fold decrease in circulating melatonin level of bipedal C57Bl/6j OPN KO mice when compared to wild-type ones (C57Bl/6j, C57Bl/6j and FVB).

[00140] As indicated above, C57Bl/6j mice are melatonin deficient and may develop a scoliosis (S) in contrast to the FVB strain, which produces high melatonin levels. OPN-knockout mice do not develop a scoliosis (NS) even if they are in the same genomic background (C57Bl6/j), although melatonin is markedly decreased, suggesting that melatonin negatively regulates OPN expression and synthesis *in vivo*. Without being bound by this hypothesis, it is also suggested that in absence of OPN in genetically modified mice, the melatonin level will be further decreased accordingly as an adaptive physiological response to enhance OPN expression and synthesis.

EXAMPLE 5

Effect of OPN inhibitors on scoliosis prevention

[00141] Two compounds suspected of having an effect on OPN transcription or synthesis were injected intraperitonealy at a dosage of 500µg/kg of body weight/day to chicken 24-48h prior pinealectomy.

[00142] As is apparent in Figure 4, fewer pinealectomized chicken pre-treated with the drugs developed scoliosis (a reduction of 50%) than untreated pinealectomized chickens.

EXAMPLE 6

Comparing the level of circulating OPN in AIS patients classified in two groups and healthy controls

[00143] A group of 252 patients with AIS and 35 healthy control subjects were tested as described in Example 1 above. Patients with AIS were divided into two subgroups according to their spinal curve severity (10°-44° vs. ≥45°) In the most severely affected AIS subgroup, none of the patients had corrective surgery at the time of the tests. Consistent with literature reporting increased AIS prevalence in teenage girls when compared to boys for moderate curves (ratio 10:1 for curve with a Cobb's angle ≥ 30°), a greater proportion of girls in the AIS groups (86% and 84%in the 10° -44° and ≥45° subgroups, respectively were observed compared to the control groups (54% and 64% in healthy and at risk control groups, respectively, $p \leq 0.0001$ when comparing the control groups). There was no significant gender difference between the two AIS subgroups (p=0.76) or between the two control groups (p=0.32). Mean age was significantly higher

in AIS patients with Cobb's angle ≥45° compared to those with 10-44° angle (15.2 ± 1.8 vs. 13.8 ± 2.1, $p<0.0001$). Both AIS groups had higher mean age compared to control groups (10.7 ± 0.6 for the healthy and 9.9 ± 3.4 for the at risk group, $p<0.0001$ when comparing to either AIS group).

[00144] The plasma OPN levels in patients with AIS exhibiting a severe deformity (Cobb's angle ≥ 45°), low to moderate curve (Cobb's angle between 10° and 44°) and healthy controls are summarized in Table 4 below according to various clinical parameters. The mean plasma OPN levels were significantly higher in both AIS groups when compared to healthy control group although plasma OPN levels were more elevated in patients with the most severe deformities (Cobb's angle ≥ 45°) (Bonferroni-corrected $p<0.001$ after adjustment for age, gender, and age-gender interaction). Plasma OPN levels in AIS patients were correlated with the severity of curve deformity (Figure 5D) in girls and boys (Partial Pearson correlation coefficient adjusted for age = 0.29, $p < 0.001$, and 0.33, $p = 0.04$, respectively). Mean plasma OPN levels in the group at risk of developing scoliosis (846 ± 402 ng/ml) differed significantly (Bonferroni-corrected $p<0.001$) from the healthy controls (570 ± 156 ng/ml).

Table 4. Mean biochemical values of patients with AIS, healthy control subjects and asymptomatic at risk control subjects*.

		Female			Male			Female + Male			
	Subject Type	N	Mean biomarker level (ng/ml)	Range	N	Mean biomarker level (ng/ml)	Range	N	Mean biomarker level (ng/ml)	Range	P-value†
OPN	Healthy controls	19	580 ± 150	318 – 882	16	558 ± 168	308 – 856	35	570 ± 156	308 – 882	–
	At risk controls	45	829 ± 419	208 – 1834	25	877 ± 378	391 – 1629	70	846 ± 402	208 – 1834	< 0.001
	AIS < 45°	162	774 ± 268	373 – 1585	27	948 ± 335	445 – 1668	189	799 ± 284	373 – 1668	< 0.001
	AIS ≥ 45°	53	913 ± 398	201 – 1821	10	1238 ± 409	575 – 1872	63	965 ± 414	201 – 1872	< 0.001
sCD44	Healthy controls	19	522 ± 99	373 – 829	16	575 ± 92	404 – 800	35	546 ± 98	373 – 829	–
	At risk controls	45	508 ± 96	316 – 760	25	533 ± 98	304 – 510	70	517 ± 97	304 – 760	> 0.5
	AIS < 45°	162	503 ± 161	194 – 1253	27	527 ± 110	364 – 793	189	506 ± 155	194 – 1253	> 0.5
	AIS ≥ 45°	53	436 ± 251	87 – 882	10	402 ± 216	147 – 962	63	431 ± 245	87 – 962	0.066
HA	Healthy controls	19	128 ± 38	72 – 236	16	132 ± 49	80 – 255	35	130 ± 43	72 – 255	–
	At risk controls	45	119 ± 51	36 – 257	25	117 ± 52	33 – 226	70	118 ± 51	33 – 257	> 0.5
	AIS < 45°	162	112 ± 60	18 – 356	27	124 ± 60	27 – 283	189	114 ± 60	18 – 356	> 0.5
	AIS ≥ 45°	53	93 ± 40	32 – 222	10	128 ± 71	41 – 25435	63	98 ± 48	32 – 254	0.140

*SD is standard deviation

† P-value is from the comparison with healthy control group in all subjects after Bonferroni correction and adjustment for age, gender, and age-gender interaction (OPN and HA) or age (sCD44). After the same adjustments, overall F test p-values for association between group and biomarker levels were < 0.001 (OPN), 0.035 (sCD44), and 0.163 (HA).

[00145] Receiver-operating characteristics (ROC) curves analyzes of plasma OPN comparing the patients with AIS more severely affected (Cobb's angle ≥45°) with healthy controls showed an AUC of 0.94 with a standard error of 0.03 (95 percent confidence interval 0.88 to 0.99) (see Figure 5A). A cut-off value > 700 nanograms per milliliter gave a sensitivity of 90.6 percent and a specificity of 81.8 percent with (see Figure 5B). A cut-off value >800 nanograms per milliliter had the highest accuracy with a sensitivity of 84.4 percent and specificity of 90.6 percent for confirming scoliosis (minimal false negative and false positive results) (see Figure 5C).

[00146] Although as indicated above, high levels of OPN are found in other adult diseases, high plasma OPN levels found in patients with scoliosis are unique in the pediatric population. The detection of OPN level can thus be used to identify within asymptomatic children those who are at risk of developing a scoliosis (AIS or other spinal disorders and disorders causing scoliosis) and identify among scoliotic subjects, those or are at risk of experiencing a progression of scoliosis. Moreover, plasma OPN levels found in AIS patients were often higher than those measured in adult diseases. OPN levels can also be used to predict the risk in adults (e.g. degenerative scoliosis and idiopathic scoliosis that progress through adulthood). Certain mutations have already been associated with other disorders that may lead to scoliosis. In a particular embodiment, the OPN levels could be used in combination with the detection of these mutations.

EXAMPLE 7

Comparing the level of circulating OPN in asymptomatic children at risk and healthy controls

[00147] A group of 70 asymptomatic children at risk of developing a scoliosis and 35 healthy control subjects were tested as described in Example 1 above. The mean plasma OPN levels in the group at risk of developing a scoliosis (846.30 ± 402 nanograms per milliliter) differed significantly (p=0.001) from the healthy controls (570 ± 156 nanograms per milliliter) and both groups were age- and gender-matched. No significant gender difference was observed (see Table 4 above).

[00148] Using a cut-off value of 800 nanograms per milliliter, it was observed that 47.9 percent of asymptomatic children in that group were above this plasma OPN value while only 8.6 percent of healthy controls were above this value. These results are in

agreement with previous reports showing that the offspring of at least one affected parent develops more often a scoliosis than ones born from unaffected parents (34, 35).

[00149] An enzyme-linked immunosorbent assay (ELISA) or RIA for OPN for instance can thus be used for early identification of subjects at risk of developing a scoliosis for purposes of prognosis and/or scoliotic patients stratification for early bracing and less-invasive surgeries with novel fusionless devices, for pharmacological treatments and to monitor responses to treatment in patients with AIS.

EXAMPLE 8

Comparing the level of circulating sCD44 in AIS patients classified two groups and healthy controls

[00150] Experiments were conducted as described in Example 1 above. The plasma sCD44 and HA levels in healthy controls, both AIS groups and asymptomatic at risk children are displayed in Table 4 above. Comparison among all groups showed no significant change in mean plasma sCD44 and HA values. However, AIS patients exhibiting the most severe spinal deformities (≥ 45°) had also the lowest mean plasma sCD44 level when compared to the other three groups (p =0.066).

[00151] CD44 and sCD44 can act as a receptor and decoy receptor for OPN respectively. In spite that no significant changes were measured among all groups tested, the most severely affected AIS patients (≥ 45°) showed the lowest mean sCD44 value among all groups tested., Interestingly, decreased plasma sCD44 levels were found in immunodeficiency and autoimmune diseases[35-37], but none of these conditions normally lead to scoliosis in absence of high plasma OPN levels, suggesting that sCD44 could play a role in AIS as disease-modifying factor by interfering with the action of OPN (see Figure 17).

EXAMPLE 9

Profiles of change in OPN levels, sCD44 levels, and Cobb's angle of AIS patients over time

[00152] The progression of biomarkers (OPN and sCD44 levels) and Cobb's angle was measured over follow up time in AIS patients. Figure 7 presents these progression in 4 selected AIS female patients (not under brace treatment) aged 12 (red), 14 (green

and blue), and 17 (yellow) at baseline visit.

[00153] Figure 8 presents the distribution of total change in OPN (left panel) and sCD44 (right panel) levels over follow-up time in AIS patients with worsened curve deformity (total increase in Cobb's angle greater than 3°) and in those without significant change in curve (no change in Cobb's angle, decrease, or increase smaller than 3°; also presents for all Average change in OPN levels was significantly higher in the group with worsened curve deformity (Wilcoxon rank sum test $p<0.01$). No significant difference was detected for sCD44 ($p>0.5$). Length of follow-up time was similar between the 2 groups ($p>0.5$).

[00154] Figure 9 shows OPN progression correlated with Cobb's angle progression in a group of AIS patients while Figure 10 shows OPN regression or stabilization correlated with Cobb's angle regression or stabilization in other AIS patients;

[00155] OPN level can be used to identify among pre-diagnosed patients those in which scoliosis will progress.

EXAMPLE 10

Profiles of change in OPN levels, sCD44 levels, and Cobb's angle of asymptomatic at risk patients over time

[00156] Figure 11 shows profiles of change in OPN and sCD44 levels angle in 4 selected at risk subjects without scoliosis: one male aged 13 (green), and 3 female aged 5 (gold), 11 (blue), and 9 (red) at baseline visit. Significant inter-subject variability was observed in the baseline levels of biomarkers and change over time among at risk subjects (especially for OPN), indicating the potential of using this biomarker as a tool to monitor onset of scoliosis in at risk subjects.

[00157] Tables 5 to 8 below present the clinical and biochemical profiles in detail for each of the healthy control subjects (Table 5), of the AIS patients with Cobb's angles of less than 45 degrees (Table 6), of the AIS patients with Cobb's angles 45° or more (Table 7), and of the asymptomatic at risk children (Table 8).

Table 5. Clinical and biochemical profile of healthy control subjects.

Random	Date of Birth	Gender	Age	Collection Date	Timepoint (months)	[OPN] (ng/ml)	[sCD44] (ng/ml)	[HA] (ng/ml)
1	1996-03-21	M	11.2	2007-05-22	T0	663.92 ± 26.03	533.4	164.87 ± 6.05
2	1996-06-26	M	10.9	2007-05-22	T0	418.23 ± 12.49	504.38	120.49 ± 2.06
			11.6	2008-01-16	T8	593.64 ± 28.77	555.88	150.02 ± 15.74
3	1996-05-28	F	11.0	2007-05-22	T0	629.52 ± 0.64	829.35	140.89 ± 3.90
			11.7	2008-01-16	T8	892.76 ± 1.54	507.54	146.71 ± 24.69
4	1996-06-22	M	10.9	2007-05-22	T0	458.68 ± 11.40	799.57	100.98 ± 6.89
5	1996-10-13	F	10.6	2007-05-22	T0	459.33 ± 2.90	525.76	139.84 ± 2.89
			11.3	2008-01-16	T8	464.46 ± 2.29	476.43	157.36 ± 20.10
7	1996-08-08	F	10.8	2007-05-22	T0	691.18 ± 2.50	664.38	120.69 ± 2.79
			11.5	2008-01-16	T8	825.38 ± 1.16	545.85	180.39 ±42.55
8	1996-02-01	M	11.3	2007-05-22	T0	498.86 ± 0.66	643.38	99.24 ± 2.35
			12.0	2008-01-16	T8	469.87 ± 11.47	440.44	154.20 ± 2.53
9	1997-06-28	M	9.9	2007-05-22	T0	517.11 ± 53.44	582.66	134.43 ± 6.42
10	1997-07-23	F	9.8	2007-05-22	T0	756.24 ± 23.61	499.03	131.04 ± 1.98
			10.5	2008-01-16	T8	1039.80 ± 3.10	337.33	167.84 ± 2.48
11	1996-02-22	M	11.3	2007-06-06	T0	653.09 ± 15.14	581.14	191.13 ± 17.98
			11.8	2007-12-04	T6	521.00± 5.82	861.46	265.54 ± 6.97
12	1996-02-09	F	11.3	2007-06-06	T0	449.97 ± 11.21	490.25	112.71 ± 17.95
			11.8	2007-12-04	T6	923.12± 1.03	476.09	188.80 ± 15.17
13	1996-05-17	F	11.1	2007-06-06	T0	488.30 ± 0.80	428.77	168.61 ± 9.49
			11.6	2007-12-04	T6	659.35 ± 1.68	584.96	182.09 ± 13.74
14	1995-10-20	M	11.6	2007-06-06	T0	610.77 ± 8.93	573.88	128.40 ± 6.58
			12.1	2007-12-04	T6	469.87 ± 19.12	527.07	167.16 ± 44.48
16	1997-03-07	F	10.2	2007-06-06	T0	544.82 ± 7.91	516.6	132.83 ± 2.07
			10.7	2007-12-04	T6	723.88 ± 8.56	503.74	65,43 ± 9,60
17	1996-05-09	M	11.1	2007-06-06	T0	450.87 ± 6.41	553.26	255.19 ± 14.61
			11.6	2007-12-04	T6	530.37 ± 16.78	267.86	42,33 ± 7,47
18	1997-09-02	F	9.8	2007-06-06	T0	555.41 ± 32.17	498.65	127.24 ± 10.65
19	1996-11-04	M	10.6	2007-06-06	T0	314.85 ± 9.93	682.71	175.92 ± 16.20
20	1997-05-30	F	10.0	2007-06-06	T0	381.57 ± 4.61	373.01	87.65 ± 3.71
			10.5	2007-12-04	T6	434.48 ± 5.73	497.7	142.61 ± 8.42
21	1997-01-07	F	10.4	2007-06-06	T0	318.19 ± 6.62	474.59	235.76 ± 3.68

			10.9	2007-12-04	T6	393.98 ± 3.87	571.14	209.26 ± 2.40
22	1997-02-09	F	10.3	2007-06-06	T0	882.15 ± 18.31	542.95	131.86 ± 1.13
			10.8	2007-12-04	T6	804.46	593.61	120.43 ± 14.60
23	1997-03-02	M	10.3	2007-06-06	T0	307.71 ± 4.88	621.23	157.12 ± 2.29
24	1997-06-19	F	10.0	2007-06-06	T0	423.06 ± 13.90	561.28	149.88 ± 5.65
25	1997-04-12	F	10.1	2007-06-06	T0	758.88 ± 5.74	478.79	169.32 ± 8.25
26	1997-12-02	M	9.5	2007-06-06	T0	441.36 ± 8.32	645.84	148.32 ± 16.36
27	1996-04-03	F	11.2	2007-06-06	T0	794.21 ± 5.50	545.62	77.58 ± 8.87
			11.7	2007-12-04	T6	748.79 ± 7.61	575.46	228.08 ± 27.64
28	1995-09-30	F	11.7	2007-06-12	T0	503.25 ± 8.16	451.68	71.91 ± 4.23
29	1996-09-15	M	10.7	2007-06-12	T0	576.62 ± 5.29	554.79	80.24 ± 3.69
			11.2	2007-12-04	T6	552.15	598.79	108.09 ± 16.44
30	1996-01-18	F	11.4	2007-06-12	T0	578.62 ± 0.24	634.22	126.21 ± 4.18
			11.9	2007-12-04	T6	498.67 ± 8.60	606.57	192.18 ± 31.90
31	1996-08-24	F	10.8	2007-06-12	T0	531.91 ± 4.36	432.2	132.19 ± 5.06
			11.3	2007-12-04	T6	455.46 ± 4.85	660.14	244.46 ± 3.49
32	1997-04-19	F	10.1	2007-06-12	T0	611.32 ± 6.46	481.47	92.69 ± 2.87
			10.6	2007-12-04	T6	406.38 ± 19.28	415.61	142.80 ± 25.25
33	1997-04-21	M	10.1	2007-06-12	T0	543.15 ± 7.32	403.56	91.82 ± 4.49
			10.6	2007-12-04	T6	360.77 ± 9.93	544.36	81.68 ± 23.85
34	1995-11-15	M	11.6	2007-06-12	T0	856.07 ± 3.82	501.71	96.37 ± 4.15
			12.1	2007-12-04	T6	922.12 ±20.68	535.71	56.34 ± 1.86
35	1996-04-22	F	11.1	2007-06-12	T0	659.81 ± 5.54	502.09	87.90 ± 4.85
			11.6	2007-12-04	T6	596.77 ± 10.14	378.46	242.42 ± 36.30
36	1995-12-09	M	11.5	2007-06-12	T0	816.64 ± 14.56	502.85	83.26 ± 0.12
37	1995-10-07	M	11.7	2007-06-12	T0	805.92 ± 14.01	511.63	80.24 ± 3.69
			12.2	2007-12-04	T6	304.61 ± 14.94	489.06	141.51 ±21.50

* Plus–minus values are means ± standard deviations.

† Healthy control subjects have no family history of scoliosis and are examined before sample collection by an orthopaedic surgeon.

Table 6. Clinical and biochemical profiles of AIS patients with Cobb's angles less than 45°.

Patient ID	Date of Birth	Gender	Age	Collection Date	Timepoint (mths)	Cobb's Angle Pre-op	Curve Type	Date of surgery	Family history	[OPN] (ng/ml)	[sCD44] (ng/ml)	[HA] (ng/ml)
102	1991-09-12	F	13.8	2005-06-10	T0	18	rT	—	Cousin	1265.10	375.56	132.06 ± 39.35
			14.3	2006-01-13	T7	16	rT			766.80	408.06	388.93 ± 23.42
			15.8	2007-06-01	T12	16	rT			933.77 ± 13.23	437.55	71.91 ± 4.23
			16.2	2007-11-30	T29	17	rT			591.72 ± 66.49	311.40	27.92 ± 1.72
103	1991-09-04	M	13.8	2005-06-10	T0	13	lT	—	Father (cyphose)	1338.32	792.62	207.12
104	1992-01-29	F	13.4	2005-06-10	T0	21-22	rTlL	—	—	1221.83	742.48	132.24
106	1992-08-10	F	14.8	2007-06-05	T0	25-24	rTlL	—	—	972.87 ± 16.73	488.72	86.78 ± 6.34
			15.2	2007-10-05	T4	22-18	rTlL			485.82 ± 34.70	475.13	293.05 ± 40.93
107	1991-09-09	F	13.8	2005-06-20	T0	31-32	rTlL	—	Mother	739.61	1253.3	109.39 ± 26.70
113	1995-11-21	F	9.7	2005-07-22	T0	10	rT	—	—	670.49 ± 5.45	695.21	41.10 ± 8.51
			11.5	2007-05-18	T22	15	rT			688.49 ± 23.78	613.79	49.16 ± 9.14
118	1991-06-04	F	16.6	2008-01-18	T0	22-22	rTlTL	—	Both parents	372.79 ± 10.86	273.31	70.42 ± 4.85
123	1993-09-23	F	12.1	2005-11-04	T0	28	rTL	—	Both parents	1466.97	931.05	128.78 ± 4.22
			14.3	2008-01-18	T26	19-31	lTrTL			779.90 ± 16.68	410.10	179.52 ± 21.17
124	1990-12-09	F	14.9	2005-11-04	T0	33-32	rTlTL	—	Cousins	625.97	816.60	96.08
127	1992-01-18	F	13.9	2005-12-02	T0	33-19	rTrT	—	—	786.71	755.60	131.36 ± 22.43
128	1997-03-18	F	8.8	2005-12-02	T0	10	lTL	—	—	837.64	628.74	118.73 ± 10.43
130	1991-06-05	F	14.5	2005-12-09	T0	19	rTL	—	—	559.85	552.78	75.09 ± 7.11
131	1992-11-09	F	13.1	2005-12-09	T0	32-24	rTlL	—	—	568.01	578.96	101.00 ± 11.04
			15.0	2007-11-12	T23	32-24	rTlL			450.45 ± 9.36	505.94	100.03 ± 9.66
136	1989-10-10	F	16.3	2006-01-13	T0	14	lTL	—	—	411.02	670.31	84.81 ± 2.56
138	1993-06-04	F	12.7	2006-02-17	T0	24-26	rTlL	—	Cousin	577.78	293.51	63.86 ± 4.11
			14.3	2007-10-24	T20	22-25	rTlL			379.04 ± 18.07	388.16	86.23 ± 11.26
			14.7	2008-02-04	T24	23-26	rTlTL			529.70 ± 4.86	378.03	227.26 ± 0.94
139	1993-12-06	F	12.2	2006-02-24	T0	12.-14	rTlL	—	—	847.98	868.95	136.19 ± 7.63
			14.2	2008-02-08	T24	12.-6	rTlL			1192.61 ± 10.71	444.33	73.88 ± 19.39
141	1992-07-20	F	13.7	2006-03-10	T0	20-18	rTlL	—	Grand-mother, cousins, uncle	658.28	735.50	90.51
			15.5	2008-01-22	T22	9.-13	rTlTL			172.67 ± 8.59	433.6	37.31 ± 7.61
142	1992-12-19	F	13.2	2006-03-10	T0	31	lTL	—	Mother, cousin	776.43	907.96	122.73 ± 7.61
			15.1	2008-01-23	T22	25	lTL			542.85 ± 1.41	511.4	146.43 ± 63.23
146	1990-05-13	F	16.0	2006-05-26	T0	32-22	rTlL	—	—	1501.42	475.91	75.68 ± 10.22
148	1993-08-12	F	14.3	2007-12-07	T0	11	lTL	—	Mother	1416.91 ± 41.50	550.4	37.79 ± 6.19

149	1988-09-28	M	17.7	2006-06-02	T0	31-26	rTIL	—	—	472.61	559.97	138.95 ± 7.42
150	1992-10-16	F	13.6	2006-06-02	T0	25	rT	—	Sister	805.88	543.22	71.24 ± 1.52
151	1993-04-11	F	14.7	2007-12-03	T0	28-20	rTIL	—	—	732.19 ± 2.30	403.51	20.80 ± 3.30
152	1990-10-04	F	15.7	2006-06-02	T0	34	IL	—	Father	655.10	551.24	122.69 ± 0.10
154	1989-11-24	F	16.6	2006-06-08	T0	40	ITL	—	Cousin	541.07	639.52	104.09 ± 13.96
			18.1	2007-12-07	T18	38	ITL			1101.07 ± 38.84	342.17	35.08 ± 5.40
155	1991-01-01	F	15.4	2006-06-08	T0	26	ITL	—	Aunt	738.59	796.06	121.33 ± 17.72
159	1998-03-04	F	9.7	2007-11-06	T0	3	ITL	—	Mother	769.50 ± 21.57	831.18	107.5 ± 1.08
161	1994-04-27	F	13.6	2007-11-30	T0	15	ITL	—	—	487.11 ± 29.43	355.79	23.63 ± 0.53
165	1995-08-30	F	12.3	2007-12-03	T0	34-20	rTIL	—	—	1148.04 ± 47.51	607.43	42.39 ± 7.68
168	1992-04-24	F	14.2	2006-06-26	T0	16-18	rTIL	—	—	810.21 ± 28.48	244.4	103.10 ± 10.39
			14.6	2006-11-21	T5	17-16	rTIL			582.52 ± 23.29	338.03	99.20 ± 18.18
			15.5	2007-10-01	T16	14-16	rTITL			441.81 ± 7.29	333.4	126.96 ± 1.45
176	1992-10-24	F	13.8	2006-07-03	T0	29	rT	—	—	503.88 ± 35.81	331.65	91.50 ± 21.99
			14.2	2007-01-15	T6	27	rT			675.38 ± 44.20	305.92	193.26 ± 2.38
183	1991-09-13	M	14.8	2006-05-07	T0	17	rL	—	—	733.99 ± 17.33	550.24	72.91 ± 10.68
			15.4	2007-06-02	T13	7.-19	rTIL			781.03 ± 3.27	531.96	69.83 ± 7.07
200	1992-07-29	M	15.2	2007-10-30	T0	23-24	rTIL	—	—	972.10 ± 4.92	401.94	88.41 ± 10.08
201	1992-11-27	F	13.7	2006-07-12	T0	10-17.	rTIL	—	Sister	782.77 ± 2.63	498.93	142.57 ± 44.69
225	1994-05-09	F	12.2	2006-07-24	T0	15-19	ITrTL	—	—	406.67 ± 3.40	617.37	248.10 ± 24.21
			12.8	2007-02-27	T7	13-18	ITrL			651.89 ± 21.69	524.9	47.95 ± 3.60
234	1990-07-16	M	16.2	2006-10-13	T0	26	rT	—	—	840.88 ± 1.98	491.26	89.04 ± 5.66
235	1991-10-29	M	15	2006-10-13	T0	20	ITL	—	—	586.25 ± 0.32	403.8	181.655 ± 48.71
			16	2007-10-11	T12	18	ITL			523.39 ± 9.76	428.29	188.63 ± 6.83
240	1993-10-04	F	13.2	2006-12-11	T0	17-23	rTIL	—	Mother, brother, cousin	525.88 ± 7.74	428.83	71.91 ± 4.23
242	1989-09-12	F	17.3	2007-01-12	T0	6	ITL	—	Sister	590.13 ± 6.00	435.59	80.24 ± 3.69
244	1990-10-20	F	16.2	2007-01-19	T0	27-29	rTIL	—	—	735.26 ± 4.42	510.44	73.81 ± 6.20
			17.3	2008-02-13	T13	NA	NA			1293.68 ± 36.92	449.1	44.51 ± 4.81
245	1992-01-27	F	15.0	2007-01-22	T0	31-35	rTIL	—	—	496.26 ± 3.54	333.97	70.41 ± 0.88
			15.8	2007-11-14	T10	28-35	rTIL			363.60 ± 2.97	562.52	54.98 ± 5.08
247	1994-12-18	F	12.1	2007-01-26	T0	9	rTL	—	Mother, sister	1148.31 ± 2.17	371.29	164.68 ± 23.99
			12.8	2007-10-09	T9	6	rTL			806.91 ± 16.69	393.27	141.16 ± 2.62
248	1997-06-16	F	9.6	2007-01-26	T0	9	rL	—	Mother, sister	1010.38 ± 5.14	443.83	142.95 ± 4.69
			10.3	2007-10-09	T9	3	ITL			841.24 ± 18.47	490.2	158.10 ±33.95

249	1991-03-25	F	15.9	2007-02-02	T0	31	ITL	—	—	534.09 ± 7.74	459.52	74.98 ± 0.08
			16.4	2007-08-03	T6	NA	ITL			340.44 ± 12.89	499.97	132.91 ± 37.20
			16.9	2008-02-01	T12	36	ITL			579.65 ± 8.62	413.67	98.93 ± 19.98
250	1992-05-08	F	14.7	2007-02-02	T0	32	ITL	—	Uncle	688.35 ± 9.46	587.17	74.40 ± 3.75
			15.4	2007-10-15	T8	21	ITL			612.19 ± 22.36	540.29	150.73
251	1991-09-05	F	15.4	2007-02-02	T0	40-30	rTIL	—	—	1146.66 ± 7.34	437.25	80.50 ± 5.24
253	1992-10-18	M	14.3	2007-02-27	T0	31	rT	—	—	634.83 ± 0.90	486.03	184.50 ± 20.76
254	1991-12-11	F	15.2	2007-03-09	T0	28	ITL	—	—	701.23 ± 1.92	362.22	72.85 ± 2.66
			15.9	2007-11-12	T8	15	ITL			548.26 ± 25.55	538.63	83.17 ± 0.07
256	1996-03-19	F	11.0	2007-03-09	T0	11	ITL	—	—	575.73 ± 5.49	530.67	97.73 ± 3.00
257	1995-04-15	F	11.9	2007-03-09	T0	6	rTL	—	Mother	995.77 ± 8.22	468.59	94.49 ± 8.02
			12.5	2007-10-16	T7	NA	NA			879.54 ± 20.53	421.24	102.11± 5.69
258	1990-06-24	M	16.8	2007-03-09	T0	14	rT	—	—	876.44 ± 9.21	564.15	89.36 ±4.66
			17.3	2007-10-02	T8	NA	NA			520.58 ± 8.52	483.28	175.81 ± 53.68
259	1994-07-07	F	12.7	2007-03-16	T0	8	ITL	—	—	1095.11 ± 7.88	397.45	85.33 ± 4.07
			13.5	2007-10-15	T7	11	ITL			1050.58 ± 5.08	466.58	139.86 ± 15.48
260	1994-07-07	M	12.7	2007-03-16	T0	6	rTL	—	—	1084.13 ± 1.82	480.1	127.84 ± 8.13
			13.5	2007-10-05	T7	4	ITL			494.25 ± 22.05	401.01	188.45 ± 31.29
261	1997-06-19	F	9.7	2007-03-16	T0	21	IL	—	—	745.79 ± 22.70	568.33	122.95 ± 2.89
			10.3	2007-10-17	T7	10	ITL			1150.38 ± 5.64	506.72	206.45± 14.75
			10.4	2008-02-06	T11	5	ITL			852.44 ± 31.69	432.45	142.46 ± 27.89
263	1994-10-13	F	12.4	2007-03-20	T0	7.-12	rTIL	—	—	989.52 ± 4.54	617.16	74.05 ± 5.38
264	1992-05-24	F	14.8	2007-03-20	T0	23-30	rTIL	—	Uncle	579.22 ± 9.53	580.38	100.39 ± 2.76
265	1993-05-04	F	13.9	2007-03-20	T0	23	IL	—	—	696.52 ± 8.57	491.96	105.88 ± 7.86
			14.5	2007-11-13	T8	11-14.	rTIL			848.34 ± 8.38	531.14	106.80 ± 1.16
266	1991-01-25	F	16.2	2007-04-02	T0	34	rTL	—	—	728.63 ± 5.47	462.66	78.08 ± 1.06
			16.8	2007-11-15	T7	34	rTL			392.63 ± 9.28	349.34	73.67 ± 3.30
267	1994-05-14	F	12.9	2007-04-02	T0	5	rTL	—	—	809.78 ± 2.39	579.14	70.57 ± 2.92
			13.5	2007-11-15	T7	5	rTL			925.13 ± 23.50	827.31	59.18 ± 8.22
268	1994-08-17	F	12.6	2007-04-04	T0	12.-4	rTIL	—	Mother	750.67 ± 17.49	385.93	107.96 ± 12.28
271	1994-11-17	F	12.4	2007-04-13	T0	23	rTL	—	—	925.40 ± 10.01	482.89	87.43 ± 12.34
			12.9	2007-10-15	T6	24	rTL			1087.79 ± 22.62	423.61	186.49 ± 10.22
272	1994-04-14	F	13.0	2007-04-13	T0	22-24	rTIL	—	Aunt	634.87 ± 15.77	531.54	86.12 ± 1.03
			13.6	2007-12-05	T8	14-15	rTIL			515.84 ± 13.88	594.47	30.80 ± 7.99
273	1991-06-30	F	15.8	2007-04-13	T0	25	rTL	—	—	455.86 ± 7.52	548.8	91.21 ± 10.34

274	1990-02-28	F	17.1	2007-04-17	T0	11.-22	rTIL	—	—	856.81 ± 23.09	461.61	103.50 ± 8.99
275	1996-04-08	F	11.0	2007-04-19	T0	27-1.	rTIL	—	—	943.57 ± 8.27	469.65	66.73 ± 5.64
			11.5	2007-10-15	T6	26-19	rTITL			339.71 ± 8.66	513.42	159.78 ± 30.24
276	1994-09-26	F	13.1	2007-10-15	T0	19-19	rTIL	—	—	430.84 ± 16.02	431.09	234.52 ± 26.95
277	1994-11-02	F	12.4	2007-04-19	T0	12	IL	—	—	724.67 ± 0.64	394.65	96.43 ± 0.04
			13.0	2007-11-14	T7	15-13	rTIL			634.03 ± 28.77	659.6	127.07 ± 4.00
278	1992-06-08	M	14.9	2007-05-04	T0	22-14	rTIL	—	Mother	1045.58 ± 1.10	364.31	106.88 ± 8.57
			15.3	2007-10-23	T5	26-28	rTIL			1118.55 ± 3.48	457.48	234.68 ± 24.37
279	1998-09-22	F	8.7	2007-05-30	T0	19	rT	—	—	978.20 ± 17.94	442.08	85.62 ± 0.14
			9.2	2007-10-05	T5	8	rT			851.57 ± 67.60	573.28	64.64
280	1992-12-18	F	14.4	2007-05-30	T0	19	rT	—	Grand-parents	839.91 ± 4.88	415.23	82.19 ± 6.30
			14.9	2007-11-02	T6	24	rTL			930.08 ± 11.55	468.35	63.88 ± 1.83
281	1994-10-17	F	12.6	2007-06-01	T0	11	rT	—	—	991.09 ± 2.95	522.65	151.89 ± 1.15
			13.1	2007-11-09	T5	9	ITL			655.22 ± 54.74	505.44	112.65 ± 14.80
282	1997-09-30	F	9.7	2007-06-13	T0	20	rT	—	—	732.03 ± 19.20	547.53	138.06 ± 12.04
			10.3	2008-01-30	T7	NA	NA			1196.46 ± 21.91	487.63	129.70 ± 7.80
286	1994-06-01	F	13.3	2007-09-17	T0	28	ITL	—	—	499.69 ± 1.97	400.19	130.85 ± 3.82
287	1991-11-15	F	15.8	2007-09-18	T0	11	rTL	—	—	602.68 ± 0.65	418.92	190.43
288	1996-05-13	M	11.3	2007-09-18	T0	20	IL	—	—	927.74 ± 4.10	533.37	55.21 ± 10.16
289	1992-10-23	F	14.9	2007-09-18	T0	18	rT	—	—	509.91 ± 5.91	362.72	81.33 ± 11.16
290	1993-10-02	F	14.0	2007-09-18	T0	22	rTL	—	Aunts	498.69 ± 46.68	507.71	127.53 ± 8.29
291	1992-07-10	F	20.9	2007-09-18	T0	25-31	rTIL	—	—	637.03 ± 7.11	467.8	154.54± 1.72
292	1994-01-23	F	13.7	2007-09-21	T0	20	ITL	—	Grand-mother	691.71 ± 37.30	581.43	76.54 ± 1.66
293	1993-04-03	F	14.5	2007-09-21	T0	16	rT	—	—	494.81 ± 7.56	359.46	166.11
295	1991-08-09	M	16.1	2007-09-26	T0	11.-8	rTIL	—	—	838.72 ± 39.67	405.48	159.20 ± 22.89
296	1992-04-04	F	15.5	2007-09-28	T0	15-18	ITrL	—	—	761.74 ± 25.61	494.27	237.77
297	1997-07-13	M	10.2	2007-09-28	T0	20	IT	—	Uncle	768.08 ± 6.70	515.45	100.00 ± 9.41
298	1994-11-09	F	12.9	2007-09-28	T0	18-21	rTIL	—	—	750.91 ± 16.94	348.87	290.06 ± 38.15
299	1990-03-21	F	17.5	2007-10-03	T0	33-43	rTIL	—	—	625.36 ± 6.80	306.11	135.94 ± 1.36
301	1995-02-06	F	12.7	2007-10-09	T0	13	IT	—	Grand-mother	948.83 ± 11.23	578.58	150.57 ± 4.40
302	1993-05-07	F	14.4	2007-10-09	T0	14.-12	rTIL	—	—	873.77 ± 2.17	373.31	230.66 ± 10.50
303	1991-03-29	F	16.5	2007-10-15	T0	14	ITL	—	—	767.96 ± 29.04	458.27	192.45± 10.19
304	1991-10-25	F	16.0	2007-10-16	T0	25	IT	—	Brother, father, all paternal family	493.39 ± 34.21	446.06	185.69 ± 12.07
305	1992-02-24	F	15.7	2007-10-19	T0	23	ITL	—	Mother	533.91 ± 18.09	364.52	123.23± 15.87

306	1994-09-22	F	13.1	2007-10-19	T0	13-18	rTIL	—	Mother	1016.54 ± 23.75	623.32	216.02 ± 19.04
307	1994-01-25	M	13.7	2007-10-24	T0	8-11-11.	ITrTIL	—	—	1328.92 ± 1.50	569.35	165.08 ± 16.63
308	1997-05-22	F	10.4	2007-10-26	T0	8	rTL	—	Aunts	430.39 ± 5.44	519.72	133.63 ± 11.13
309	1996-04-10	F	11.5	2007-10-26	T0	10	ITL	—	Mother, cousins	536.77± 9.30	485.45	285.92 ± 25.08
311	1993-05-07	F	14.5	2007-10-26	T0	17	ITL	—	—	493.18 ± 23.85	546.9	110.66 ± 9.59
313	1993-06-04	F	14.4	2007-10-26	T0	20-18	rTIL	—	Cousin	536.22 ± 4.65	379.49	99.52 ± 2.41
314	1993-03-11	F	14.6	2007-10-29	T0	24	rL	—	Mother	939.67 ± 37.16	549.66	78.11 ± 7.22
315	1993-12-16	F	13.9	2007-10-31	T0	14	ITL	—	—	537.59 ± 1.16	481.91	142.26 ± 23.98
316	1992-10-07	M	15.1	2007-10-31	T0	28	rT	—	—	636.17 ± 2.31	576.05	94.21 ± 5.42
318	1997-05-25	F	10.4	2007-10-15	T0	11	rTL	—	Mother	1151.62 ± 33.64	634.57	112.13 ± 23.16
319	1993-06-28	F	14.4	2007-11-06	T0	22	ITL	—	Cousin	518.10 ± 27.77	667.02	79.46 ± 6.89
320	1993-09-24	F	14.1	2007-11-09	T0	15	rT	—	—	452.54 ± 10.01	765.38	134.09 ± 21.38
321	1992-07-04	F	15.3	2007-11-09	T0	16	rTL	—	—	470.02 ± 16.75	377.13	110.37 ± 12.77
322	1996-06-01	F	11.4	2007-11-09	T0	4	ITL	—	—	565.20 ± 48.73	492.94	95.12 ± 7.44
324	1991-04-20	F	16.6	2007-11-09	T0	19-19	rTIL	—	—	659.93 ± 14.39	562.52	98.61 ± 6.25
325	1994-03-26	F	13.6	2007-11-09	T0	21	rTL	—	Mother, grand-parents	761.48 ± 3.82	846.66	89.91 ± 12.48
326	1994-02-02	M	13.8	2007-11-13	T0	13	ITL	—	—	1451.37 ± 77.12	617.35	240.72 ± 27.74
328	1994-09-24	F	12.8	2007-11-14	T0	11	ITL	—	—	580.55 ± 24.91	876.97	174.59
329	1996-05-29	F	11.5	2007-11-14	T0	6	rTL	—	Mother	877.16 ± 27.08	953.41	269.12± 4.88
330	1994-02-05	F	13.8	2007-11-16	T0	12	ITL	—	—	1403.38 ± 20.98	465.43	279.56
332	1992-01-26	M	15.8	2007-11-23	T0	24	ITL	—	—	864.14 ± 43.84	699.27	175.34 ± 30.44
333	1993-10-21	F	14.1	2007-11-23	T0	30	ITL	—	Cousin	564.09 ± 7.37	762.16	143.10 ± 30.54
334	1993-08-07	F	14.3	2007-11-23	T0	29-27	rTIL	—	—	896.91 ± 29.60	727.33	155.95 ± 38.28
335	1996-01-16	F	11.9	2007-11-23	T0	28-27	rTIL	—	—	1192.08 ± 14.98	839.56	162.32 ± 0.67
337	1991-09-04	M	16.2	2007-11-28	T0	24	IL	—	Sister	914.93 ± 10.71	788.28	114.15 ± 25.71
338	1994-12-31	F	12.9	2007-11-30	T0	10	ITL	—	Aunt	539.94 ± 1.35	301.42	38.44 ± 5.53
339	1992-03-17	F	15.7	2007-11-30	T0	25	ITL	—	Grand-father	747.48 ± 9.20	444.12	253.92
340	1995-05-21	F	12.5	2007-11-30	T0	30	ITL	—	—	746.48 ± 45.11	498.56	259.46
341	1996-02-11	F	11.8	2007-11-30	T0	15-14	rTIL	—	Cousin	947.50 ± 31.38	662.73	75.40±1.41
342	1993-12-01	F	14.0	2007-12-07	T0	16	rTL	—	—	993.33 ± 55.93	376.73	19.57 ± 5.63
343	1993-06-29	M	14.4	2007-12-07	T0	15	rTL	—	Grand-mother	996.61 ± 25.86	541.76	43.48 ± 2.96
344	1996-03-26	F	11.7	2007-12-07	T0	10	rTL	—	—	637.78 ± 7.73	702.48	26.94 ± 5.89
345	1993-04-12	F	14.6	2007-12-07	T0	30	ITL	—	Cousin	722.43 ± 18.56	429.44	31.74 ± 1.77

346	1996-10-11	F	11.2	2007-12-07	T0	18-17	rTITL	—	—	576.26 ± 24.83	436.35	29.25 ± 2.56
347	1997-04-07	F	10.7	2007-12-11	T0	5-6.	rTIL	—	Sister	1272.11± 18.19	425.98	41.20 ± 4.60
348	1995-06-10	M	12.5	2007-12-11	T0	10	rTL	—	Sister	776.87± 50.77	384.51	27.13 ± 1.84
350	1995-02-22	F	12.8	2007-12-13	T0	25	rTL	—	—	1020.59 ± 46.63	488.19	32.35 ± 2.16
351	1992-05-19	F	15.6	2007-12-13	T0	14	rTL	—	Father	557.14 ± 25.67	475.23	20.16 ± 2.76
352	1996-04-13	M	11.7	2007-12-13	T0	14	rTL	—	Father	1339.62 ± 39.88	566.82	97.02
353	1993-08-12	M	14.3	2007-12-13	T0	24	rT	—	—	1569.33 ± 43.27	607.43	105.59 ± 95.83
354	1994-06-07	F	13.5	2007-12-13	T0	8	IT	—	—	608.88 ± 6.80	431.16	69.78 ± 40.24
355	1993-08-08	F	14.3	2007-12-13	T0	27	ITL	—	—	691.05 ± 37.53	378.46	24.41 ± 12.43
356	1995-05-17	F	12.6	2007-12-13	T0	19	ITL	—	—	824.89 ± 1.39	467.45	43.63
358	1997-02-27	F	10.9	2008-01-11	T0	18	rTL	—	—	554.86 ± 8.43	387.21	116.04 ± 22.53
359	1995-11-08	F	13.0	2008-01-15	T0	14	rTL	—	—	709.63 ± 3.85	485.94	195.32 ± 34.14
360	1992-05-24	F	15.6	2008-01-15	T0	14	ITL	—	Mother	466.35 ± 12.61	335.02	157.17 ± 7.22
361	1996-06-29	F	11.5	2008-01-15	T0	23	rTL	—	Aunt	899.31 ± 10.09	441.72	81.52 ± 1.47
362	1997-08-21	F	10.4	2008-01-16	T0	11	ITL	—	Grand-mother	471.73 ± 21.57	437.35	110.36 ± 7.42
363	1993-05-24	F	14.6	2008-01-16	T0	20-24-19	ITrTITL	—	Mother, grand-mother, aunt	743.10 ± 15.01	353.53	161.77 ± 25.40
364	1995-03-24	F	12.8	2008-01-16	T0	10	ITL	—	Mother, grand-mother, aunt	767.06 ± 11.17	460.75	160.24 ± 26.97
365	1999-07-26	F	9.3	2008-01-16	T0	5	rTL	—	Mother, grand-mother, aunt	883.48 ± 2.32	403.41	127.81 ± 23.58
368	1996-07-12	F	11.5	2008-01-18	T0	14	rTL	—	—	1206.06 ± 43.70	415.24	136.62 ± 28.94
369	1992-05-21	F	15.7	2008-01-18	T0	25	rTL	—	—	454.71 ± 13.34	431.44	132.25 ± 19.69
370	1994-12-01	F	13.1	2008-01-18	T0	18-15	rTIL	—	—	855.36 ± 10.35	395.7	140.53 ± 2.77
371	1992-02-04	F	16.0	2008-01-18	T0	26-20	rTITL	—	Aunt, cousin	740.05 ± 5.38	487.74	112.07 ± 3.13
372	1991-06-21	F	16.6	2008-01-21	T0	23-21	rTIL	—	—	436.58 ± 40.88	395.61	170.65 ± 13.44
374	1992-05-26	F	15.7	2008-01-21	T0	25	IL	—	—	496.50 ± 28.07	401.4	77.69 ± 6.60
375	1992-10-21	F	15.3	2008-01-22	T0	31-55	rTITL	—	—	475.88 ± 0.00	385.69	130.95 ± 3.80
376	1993-05-18	F	14.7	2008-01-22	T0	16	rTL	—	—	554.83 ± 44.65	387.61	73.78 ± 0.15
377	1995-01-31	F	13.0	2008-01-22	T0	27	ITL	—	—	739.47 ± 8.03	384.16	79.40 ± 1.15
379	1996-09-14	F	11.4	2008-01-25	T0	5.-5	ITrTL	—	—	1404.12 ± 66.84	659.32	78.73 ± 2.62
381	1992-01-11	M	16.0	2008-01-25	T0	24	rT	—	—	782.27 ± 1.42	505.65	283.01 ± 26.97
382	1993-10-21	F	14.2	2008-01-25	T0	28-25	rTITL	—	—	998.95 ± 9.12	327.82	77.64 ± 12.98
383	1994-11-20	F	13.2	2008-01-25	T0	30-27	rTITL	—	—	900.32 ± 24.08	401.79	83.98 ± 7.31
384	1992-02-09	M	16.0	2008-01-29	T0	25-19	rTIL	—	—	479.70 ± 36.72	444.82	134.93 ± 7.83
386	1994-09-02	F	13.4	2008-02-01	T0	25-14	ITrT	—	—	732.99 ± 28.62	637.86	129.78 ± 2.15

387	1994-04-11	F	13.8	2008-02-01	T0	14-15	rTITL	—	Cousin	853.05 ± 70.97	373.81	146.21 ± 6.37
388	1995-11-24	F	12.2	2008-02-01	T0	34	rT	—	—	963.01 ± 40.86	465.02	66.49 ± 7.43
389	1997-04-13	F	10.8	2008-02-04	T0	14	ITL	—	Father	689.25 ± 35.56	435.9	67.38 ± 15.52
390	1994-04-28	F	13.8	2008-02-04	T0	28-26	rTIL	—	Father	930.28 ± 18.25	368.83	56.32 ± 0.12
391	1994-07-01	F	13.6	2008-02-05	T0	37	rTL	—	—	540.38 ± 9.17	501.81	49.99 ± 7.23
392	1998-11-25	F	9.2	2008-02-05	T0	16	rTL	—	Brother	661.55 ± 38.23	412.14	77.84 ± 23.22
393	1993-09-30	M	14.3	2008-02-05	T0	26	rTL	—	Brother	1235.01 ± 29.98	488.02	106.86 ± 17.43
395	1995-05-24	F	12.7	2008-02-08	T0	11	rT	—	Mother	716.48 ± 30.93	496.45	82.74 ± 2.92
397	1999-02-20	F	9.0	2008-02-08	T0	10	rTL	—	Mother, grand-mother	751.57 ± 2.34	543.59	85.71 ± 21.81
398	1997-09-16	F	10.4	2008-02-08	T0	16	rTL	—	Mother, grand-mother	872.92 ± 6.46	526.34	98.45 ± 6.33
399	2000-09-28	M	7.4	2008-02-08	T0	22-20	rTITL	—	—	444.55 ± 43.23	481.5	74.45 ± 10.16
400	1994-05-25	F	13.7	2008-02-08	T0	12	rTL	—	Mother, aunt	1492.58 ± 30.46	477.59	135.22 ± 2.80
401	1994-02-17	F	14.0	2008-02-18	T0	28-21	rTITL	—	—	691.24 ± 23.14	316.38	50.01 ± 1.95
402	1991-07-15	F	16.6	2008-02-14	T0	19-12	rTIL	—	—	423.93 ± 1.08	314.48	36.64 ± 2.04
403	1995-02-21	F	13.0	2008-02-14	T0	13-13	rTITL	—	Sister	1216.81 ± 131.72	354.37	52.43 ± 15.76
1264	1997-09-22	F	15.2	2005-04-18	T0	40	rTL	2005-04-18	—	616.12	578.96	65.92
1276	1997-09-23	F	15.2	2005-05-16	T0	42	IT	2005-05-16	—	817.56	450.13	107.62 ± 12.96
1364	1997-09-24	M	14.9	2006-04-24	T0	44	ITL	2006-04-24	Sister, aunt	1668.06	407.4	80.85 ± 6.90
1365	1990-05-11	F	15.9	2006-04-26	T0	23-53	ITrL	2006-04-26	—	947.35	642.66	63.18 ± 5.41
1366	1993-04-06	F	13.1	2006-05-01	T0	36	NA	2006-05-01	—	1317.97	323.04	89.70 ± 20.57
1373	1991-10-07	F	14.6	2006-05-17	T0	41-48	rTIL	2006-05-17	—	1584.54	583.14	80.12 ± 18.75
1380	1989-10-09	F	16.7	2006-06-26	T0	35	rL	2006-06-26	—	1289.98	602.35	139.38
1384	1991-01-17	F	15.5	2006-07-03	T0	41	ITL	2006-07-03	—	1502.51 ± 18.63	194.3	121.65 ± 44.94
			15.8	2006-11-15	T4	9-4				1258.85 ± 16.20	448.68	162.01 ± 11.64
1385	1990-06-12	F	16.1	2006-07-04	T0	42-23	rTIL	2006-07-04	—	1098.75	523.52	102.35
1387	1991-07-15	F	15.0	2006-07-17	T0	29-37-35	rTIL	2006-07-17	Mother	1017.47	689.52	78.42
1388	1991-12-13	F	14.6	2006-07-19	T0	38	rTL	2006-07-19	—	1080.53	811.37	87.57
1409	1993-02-11	F	13.6	2006-09-26	T0	40	rT	2006-09-26	—	499.41 ± 67.54	389.14	113.56 ± 15.03
1433	1992-07-03	F	14.5	2007-01-10	T0	44	rT	2007-01-10	Uncle	459.61 ± 17.79	287.42	263.55 ± 34.89
1451	1995-01-13	F	12.2	2007-03-14	T0	42	rT	2007-03-14	Grand-mother	1099.93 ± 48.11	290.5	158.45 ± 3.94
1478	1990-08-06	F	16.8	2007-06-11	T0	41	rTL	2007-06-11	Father	619.94 ± 46.51	251.56	190.25 ± 18.46
1481	1990-08-15	F	16.8	2007-06-18	T0	40	rT	2007-06-18	—	748.36 ± 9.30	250.14	95.34 ± 6.52
1483	1989-06-26	F	18.0	2007-06-19	T0	37-25	rTIL	2007-06-19	—	489.30 ± 93.18	396.39	167.02 ± 28.62

1487	1990-05-30	F	17.1	2007-07-03	T0	35-58-35	ICrTiL	2007-07-03	Aunts	508.82 ± 50.08	281.48	17.75 ± 1.94

* Plus–minus values are means ± standard deviations.

** All patients are diagnosed with AIS

† Curve type nomenclature: r, right/ l, left/ T, Thoracic/ L, Lumbar/ TL, Thoracolumbar/ C, Cervical.

‡ Certain clinical information may not have been available at the time of the study, NA.

Table 7. Clinical and biochemical profiles of AIS patients with Cobb's angles of 45° or more.

Patient ID	Date of Birth	Gender	Age	Collection Date	Timepoint (months)	Cobb's Angle Pre-op	Curve Type	Date of Surgery	Family History	[OPN] (ng/ml)	[sCD44] (ng/ml)	[HA] (ng/ml)
101	1988-05-22	F	17.1	2005-06-10	T0	47	rT	—	—	1047.64	728.42	221.97 ± 8.23
108	1989-08-29	F	15.9	2005-07-04	T0	45	lL	—	—	774.45	704.05	86.15 ± 12.73
			17.2	2006-11-21	T16	40	lL			414.67 ± 55.62	361.83	172.00 ± 3.68
135	1987-12-31	F	18.0	2006-01-13	T0	47-30	rTlL	—	—	657.01	839.02	117.48 ± 5.37
145	1990-02-15	M	16.2	2006-04-21	T0	50-43	rTlTL	—	Brother	1178.85	961.85	120.52 ± 8.59
170	1991-07-08	F	14.9	2006-06-26	T0	53-22	rTlL	2007-08	Aunt	480.97 ± 29.49	317.2	33.76 ± 0.92
			15.9	2007-04-18	T10	44-21	rTlL			540.63 ± 10.65	410.66	70.69 ± 4.67
1150	1992-04-18	F	12.1	2004-05-11	T0	84	rT	2004-05-11	Mother, grand-mother	884.02	874.59	97.74
1169	1989-09-19	F	14.8	2004-06-22	T0	54-52	rTlL	2004-06-22	—	776.13	868.43	101.22 ± 9.41
1192	1990-10-16	F	13.9	2004-09-08	T0	59	rT	2004-09-08	—	1140.09	596.41	66.97
1212	1991-05-06	F	13.5	2004-11-22	T0	54	rT	2004-11-22	Great-aunt	834.47	796.56	75.57
1254	1991-07-23	F	13.7	2005-03-16	T0	52-49	rTlL	2005-03-16	—	1091.92	882.29	82.8
1267	1990-09-08	F	14.6	2005-04-25	T0	55	lT	2005-04-25	—	509.48	596.41	76.87
1282	1988-12-29	F	16.5	2005-06-06	T0	49	rT	2005-06-06	—	718.45	788.41	53.95 ± 16.65
1310	1990-05-05	F	15.6	2005-11-09	T0	55-42	rTlL	2005-11-09	—	1042.25	789.32	132.89
1353	1989-08-08	F	16.6	2006-03-27	T0	46	lT	2006-03-27	—	1078.92 ± 33.32	262.59	90.88 ± 1.59
			17.2	2006-10-06	T7	2	NA			44.35 ± 0.50	342.48	157.74 ± 37.90
1354	1991-11-18	F	14.3	2006-03-27	T0	45	rT	2006-03-27	—	1378.360	725.138	61.016
1355	1990-02-26	M	16.1	2006-03-28	T0	74-53	rTlL	2006-03-28	—	1871.67	467.38	253.56 ± 6.84
1357	1990-08-23	F	14.8	2005-06-15	T0	47-50	rTlL	2006-04-04	Brother	705.92 ± 16.09	415.22	174.61 ± 74.40
			15.7	2006-04-04	T10	57-50	rTlL			1788.1	374.7	76.86 ± 4.78
1360	1996-05-09	F	9.9	2006-04-10	T0	53-46	rTlL	2006-04-10	Father, aunt	1820.95	444.42	80.45 ± 29.61
1361	1989-09-03	F	16.6	2006-04-10	T0	65-95	rTlL	2006-04-10	—	1512.16	599.64	67.13 ± 10.66
1369	1992-02-19	F	14.2	2006-05-09	T0	88	rT	2006-05-09	—	1498.66	262.58	91.42 ± 8.52
			14.8	2006-11-24	T6	25	NA			541.43 ± 10.31	317.72	166.79 ± 35.56
1371	1991-01-30	F	15.3	2006-05-15	T0	72-59	rTlL	2006-05-15	—	1723.91	224.15	89.53 ± 18.60
1372	1990-09-06	F	15.7	2006-05-16	T0	63-45-33	rTLlLC	2006-05-16	Aunt	1016.66	597.2	65.24 ± 5.40
1374	1989-10-05	F	16.6	2006-05-29	T0	45	lTL	2006-05-29	—	1698.01	544.71	70.32 ± 16.24
1378	1992-12-14	M	13.5	2006-06-05	T0	70	lTL	2006-06-05	—	1531.64	394.74	249.97
1381	1990-10-03	F	15.7	2006-06-27	T0	66	lT	2006-06-27	—	1032.61	626.25	89.25

1389	1995-10-26	F	10.7	2006-07-24	T0	46-66	rTITL	2006-07-24	—	899.76 ± 20.49	359.31	187.61 ± 62.69
			11.0	2006-10-02	T5	NA	NA			770.91 ± 13.31	533.42	82.67 ± 1.55
1390	1990-12-12	F	15.6	2006-07-24	T0	53	ITL	2006-07-24	—	1269.89	839.02	78.42
1392	1993-05-25	F	13.2	2006-07-26	T0	48	rT	2006-07-26	Grand-mother, aunts	1341.80 ± 15.38	87.13	105.48 ± 0.34
1393	1991-05-09	F	15.2	2006-07-26	T0	56	ITL	2006-07-26	—	969.63	821.21	81.59
1395	1988-10-25	F	17.8	2006-08-08	T0	84	ITL	2006-08-08	Aunt	1205.3	450.13	41.8
1396	1995-05-27	F	11.2	2006-08-14	T0	74-62	rTIL	2006-08-14	—	1624.64 ± 5.10	166.83	172.75 ± 26.23
			11.3	2006-09-26	T1	NA	NA			773.40 ± 16.42	342.29	218.18 ± 2.83
1397	1988-12-23	M	17.7	2006-08-29	T0	60-58	rTIL	2006-08-29	Uncle	1581.40 ± 11.23	440.95	106.21 ± 10.20
			17.9	2006-10-11	T2	34-23	NA			1191.01 ± 14.64	546.18	158.77 ± 21.05
1406	1991-10-29	F	14.9	2006-09-20	T0	62-60	rTIL	2006-09-20	—	628.36 ± 45.23	304.04	52.88 ± 0.66
1410	1993-01-04	F	13.7	2006-09-28	T0	56	rT	2006-09-28	Mother, aunt	1287.16 ± 3.12	133.56	119.48 ± 24.22
			13.8	2006-11-21	T2	23	NA			903.57 ± 52.88	328.75	141.76 ± 12.56
1416	1991-07-10	F	15.4	2006-11-15	T0	56-30	rTIL	2006-11-15	—	514.30 ± 15.49	233.55	121.42 ± 28.69
1420	1993-06-30	F	13.4	2006-11-29	T0	60-48	rTIL	2006-11-29	Sister, aunt	661.35 ± 21.22	314.01	127.14 ± 1.06
1422	1994-06-27	F	12.4	2006-12-06	T0	60-50	rTIL	2006-12-06	Sister	530.56 ± 6.57	190.55	61.30 ± 14.49
1430	1989-09-28	F	17.3	2007-01-03	T0	48	rT	2007-01-03	—	533.56 ± 24.89	228.54	51.29 ± 7.00
1442	1994-08-21	F	12.5	2007-02-14	T0	60	rT	2007-02-14	—	512.99 ± 44.58	163.01	162.44 ± 3.03
1446	1988-07-10	F	18.6	2007-02-26	T0	60	rT	2007-02-26	—	537.87 ± 4.70	332.42	66.44 ± 20.48
1448	1992-12-07	F	14.3	2007-03-13	T0	49	ITL	2007-03-13	—	588.73 ± 25.88	110.3	138.81 ± 10.07
1457	1993-05-30	F	13.9	2007-04-10	T0	50-43	rTIL	2007-04-10	—	1073.67 ± 69.04	401.79	83.21 ± 0.17
1458	1991-09-27	F	15.4	2007-04-11	T0	45	rT	2007-04-11	—	401.08 ± 22.88	212.16	66.48 ± 0.55
1459	1990-03-28	F	17.1	2007-04-16	T0	72-36	rTIL	2007-04-16	—	761.78 ± 11.69	104.61	42.08 ± 5.99
			17.2	2007-05-18	T1	NA	NA			744.34 ± 10.91	340.71	
1461	1990-05-17	F	16.9	2007-04-18	T0	48	rT	2007-04-18	Sister	200.53 ± 3.68	371.51	112.29 ± 27.44
1464	1990-01-02	F	17.3	2007-04-25	T0	53	rT	2007-04-25	—	778.26 ± 19.40	163.01	133.86 ± 4.16
1467	1990-11-18	F	16.5	2007-05-08	T0	60	rT	2007-05-08	—	453.32 ± 17.32	236.23	48.59 ± 6.73
1468	1991-11-12	M	15.5	2007-05-14	T0	69	rTL	2007-05-14	Cousin	574.80 ± 42.46	283.37	116.85 ± 14.54
1471	1989-10-08	F	17.6	2007-05-29	T0	60	rTL	2007-05-29	—	907.06 ± 34.13	332.42	66.91 ± 28.51
1474	1989-06-24	M	18.0	2007-06-04	T0	54-52	rTIL	2007-06-04	—	1254.39 ± 4.53	334.72	71.72 ± 16.08
1477	1992-10-17	F	14.6	2007-06-06	T0	62-65	rTIL	2007-06-06	Mother, brother	829.32 ± 15.89	355.03	150.57 ± 28.87
1484	1991-04-27	F	16.2	2007-06-26	T0	60	rT	2007-06-26	—	489.15 ± 20.09	216.67	88.54 ± 422
1488	1992-02-17	M	15.4	2007-07-16	T0	87	rT	2007-07-16	Mother	1358.23 ± 56.62	304.83	120.78 ± 13.25

1489	1990-09-26	M	16.8	2007-07-17	T0	57	rT	2007-07-17	—	1417.61 ± 0.00	146.93	135.42 ± 2.53
1495	1992-03-19	F	15.5	2007-09-17	T0	67-39	rT	2007-09-17	—	437.55 ± 14.74	227.82	32.06 ± 0.29
1498	1992-11-05	F	14.9	2007-09-18	T0	51-42	rTL	2007-09-18	—	557.43 ± 50.58	152.3	62.63 ± 12.90
1501	1989-02-04	F	16.5	2005-07-22	T0	58	rTL	—	—	939.53	711.38	144.30 ± 16.14
			17.8	2006-11-21	T16	60	rTL			580.11 ± 7.56	503.43	107.24 ± 7.29
1502	1994-03-14	F	13.6	2007-10-15	T0	55-43	rTIL	2007-10-15	—	856.14 ± 4.95	386.19	152.27 ± 5.09
			13.8	2007-12-05	T2	NA	NA			1089.57 ± 22.51	349.14	55.91 ± 10.45
1506	1992-07-07	F	15.3	2007-11-06	T0	65	rT	2007-11-06	—	675.53 ± 13.63	241.98	85.64 ± 24.87
1517	11/20/1990	M	17.2	2008-02-13	T0	50-62	rTITL	—	—	666.49 ± 65.68	328.96	41.3 ± 8.74
1518	12/8/1991	F	16.2	2008-02-13	T0	62-62	rTIL	—	—	672.59 ± 35.53	440.55	67.71 ± 6.81
1519	1993-04-19	M	14.8	2008-02-08	T0	51	rT	—	—	945.23 ± 53.53	360.02	66.48 ± 1.10
1520	1993-06-26	F	14.6	2008-02-08	T0	54-42	rTITL	—	—	752.87 ± 23.12	288.35	87.08 ± 0.36

* Plus–minus values are means ± standard deviations.

** All patients are diagnosed with AIS

† Curve type nomenclature: r, right/ l, left/ T, Thoracic/ L, Lumbar/ TL, Thoracolumbar/ C, Cervical.

‡ Certain clinical information may not have been available at the time of the study, NA.

Table 8. Clinical and biochemical profiles of asymptomatic at risk children.

Family Id	Date of Birth	Gender	Age	Collection Date	Timepoint (months)	Family History	[OPN] (ng/ml)	[sCD44] (ng/ml)	[HA] (ng/ml)
1	1997-09-02	M	8.8	2006-07-10	T0	Mother	439.72 ± 12.32	561.46	118.71 ± 8.74
1	1995-09-06	F	10.8	2006-07-10	T0	Mother	207.88 ± 0.93	315.67	180.71 ± 19.91
2	1998-02-08	F	8.7	2006-10-03	T0	Mother, uncle, grand-father	1650.21 ± 13.90	416.99	199.56 ± 55.60
			9.2	2007-04-19	T6		1966.98 ± 1.96	459.89	207.57 ± 39.18
			9.8	2007-12-12	T14		1816.83 ± 24.08	387.1	209.86 ± 21.38
2	2001-06-18	M	5.8	2007-04-19	T0	Mother, uncle, grand-father	493.98 ± 7.26	463.68	43.99 ± 3.74
			6.5	2007-12-12	T8		684.54 ± 10.06	438.94	102.21 ± 61.17
3	1994-08-24	F	12.2	2006-10-19	T0	Sister	690.58 ± 2.92	418.18	220.8
			12.6	2007-05-02	T7		727.27±17.36	467.79	196.82 ± 18.74
			13.2	2007-12-12	T14		1212.32 ± 0.48	311.06	279.74 ± 30.33
4	2003-10-17	F	3.0	2006-10-19	T0	Mother	1530.90 ± 28.42	478.58	225. 02 ± 20.51
			3.5	2007-04-11	T6		1021.07 ± 7.22	464.63	122.36 ± 15.35
			4.2	2007-12-12	T14		1594.42 ±23.36	470.05	332.11
5	2003-07-17	M	3.2	2006-10-19	T0	Mother	905.58 ± 30.14	563.44	58.88 ± 3.86
			3.7	2007-04-19	T6		1865.13 ± 7.35	434.93	128.14 ± 4.00
			4.4	2007-12-09	T14		960.14 ± 26.22	631.93	32.64 ± 5.81
6	1998-07-26	F	8.2	2006-10-19	T0	Mother	505.03 ± 8.92	564.17	81.86 ± 13.18
7	1995-06-16	F	11.3	2006-10-24	T0	Mother	548.59 ± 6.61	512.92	80.39 ± 31.53
			11.8	2007-04-11	T6		766.85 ± 5.73	396.69	103.31 ± 22.50
			12.3	2007-10-17	T12		596.91 ± 35.50	465.36	122.40± 8.97
8	1996-04-10	F	10.5	2006-10-26	T0	Mother	1109.78 ± 47.61	401.66	77.16 ± 9.72
			11	2007-04-11	T6		875.81 ± 14.01	366.36	176.96 ± 4.68
9	1995-05-09	F	11.4	2006-10-26	T0	Mother	1657.97	440.3	112.58 ± 0.45
			11.9	2007-04-11	T6		782.29 ± 1.47	429.56	86.57 ± 1.46
			12.8	2008-02-13	T16		885.10 ± 35.98	255.6	63,42 ± 7,99
10	2002-08-03	F	4.2	2006-10-26	T0	Mother	901.66 ± 12.01	398.27	158.65 ± 60.85
			4.7	2007-04-11	T6		929.42 ± 3.07	356.88	167.19 ± 0.13
11	1992-09-07	F	14.1	2006-10-26	T0	Mother	528.00 ± 8.83	469.78	69.05 ± 4.37
			14.8	2007-07-11	T9		714.79 ± 14.44	383.1	37.97 ± 3.99
			15.3	2008-01-23	T15		443.30 ± 0.58	472.69	80.27 ± 11.45
12	1991-12-15	F	14.8	2006-10-26	T0	Mother	818.88 ± 0.94	518.03	134.08 ± 84.67

			15.3	2007-04-11	T6		648.15	487.38	140.02 ± 50.63
			15.9	2007-11-14	T13		398.28 ± 19.81	521.44	191.07 ± 8.20
12	1996-02-23	M	10.7	2006-10-26	T0	Mother	1203.88 ± 55.29	681.23	85.30 ± 36.75
			11.2	2007-04-11	T6		1930.95 ± 1.96	633.37	107.10 ± 15.99
			11.8	2007-11-14	T13		1341.78 ±31.57	687.61	170.54 ± 25.46
13	1993-10-09	F	13.0	2006-10-26	T0	Mother, grand-mother	730.44 ± 33.95	397.12	41.87 ± 4.55
			13.6	2007-05-02	T7		420.91 ± 23.59	412.49	216.75 ± 27.71
			14.1	2007-11-14	T13		943.64 ± 1.96	698.95	124.28 ± 15.03
14	2001-09-07	F	5.2	2006-11-16	T0	Father	919.94 ± 11.91	510.08	45.28 ± 10.89
15	1997-02-18	M	9.8	2006-11-16	T0	Mother	1629.22 ± 12.49	611.25	129.80 ± 30.80
			10.2	2007-04-11	T5		1030.34 ± 6.55	690.56	146.19 ± 2.58
			10.7	2007-10-10	T11		929.36 ± 11.23	590.8	135.89 ±18.75
16	2002-02-21	F	4.8	2006-11-16	T0	Mother	1834.30 ± 4.16	628.94	149.05 ± 19.17
			5.2	2007-04-11	T5		909.22 ± 6.67	661.18	125.31
			5.9	2007-12-12	T13		877.48 ± 23.75	466.59	70.10 ± 33.68
17	2000-03-30	F	6.7	2006-11-16	T0	Mother	482.76 ± 10.64	678.55	95.92 ± 18.21
18	2000-08-01	F	6.2	2006-11-16	T0	Mother	870.73 ± 21.30	644.62	146.12 ± 36.68
18	1997-05-05	M	9.5	2006-11-16	T0	Mother	1123.32 ± 7.06	401.66	112.68 ± 11.34
20	1998-09-27	F	8.2	2006-11-22	T0	Father	506.21 ± 10.03	456.42	59.40 ± 30.21
			8.8	2007-07-11	T8		677.71 ± 13.95	416.28	37.11 ± 6.95
21 (015)	1998-11-17	F	8.0	2006-11-22	T0	Sister	482.63 ± 7.58	458.02	99.16 ± 5.46
			8.5	2007-05-23	T6		511.46	488.33	151.08
			9.0	2007-11-14	T12		760.00 ± 3.99	589.62	190.77± 5.64
21 (016)	1991-08-13	F	15.2	2006-11-22	T0	Sister	617.06 ± 7.65	511.71	110.15 ± 12.37
			15.7	2007-05-23	T6		619.60 ± 17.63	519.3	93.16 ± 0.39
			16.2	2007-11-14	T12		685.18 ± 0.80	529.63	218.26 ± 27.22
22	1992-05-15	M	14.5	2006-11-22	T0	Mother, grand-mother	1082.23 ± 65.01	445.66	81.35 ± 14.77
			14.9	2007-04-11	T5		1044.90 ± 3.21	432.72	152.54 ± 10.62
			15.6	2008-01-23	T14		1010.18 ± 60.70	384.16	106.42 ± 10.80
23 (334)	1994-09-24	F	12.2	2006-11-29	T0	Sister	1365.94 ± 1.71	346.45	150.14 ± 2.53
			12.6	2007-04-19	T5		1856.82 ± 12.74	501.92	167.91 ± 17.19
			13.1	2007-10-10	T11		947.97 ± 16.31	489.38	271.36± 20.40
24	1994-11-24	M	12.0	2006-11-29	T0	Mother, aunt	775.28 ± 20.77	427.49	84.54 ± 0.14
			12.5	2007-05-02	T6		610.29± 10.86	436.82	130.53 ± 2.30

			13.1	2007-12-12	T13		718.55± 5.97	355.99	127.92 ± 3.93
24	1994-11-24	F	12	2006-11-29	T0	Mother, aunt	815.81 ± 22.25	473.76	160.63 ± 8.36
			12.5	2007-05-02	T6		673.56 ± 16.29	445.36	127.40 ± 37.13
			13.1	2007-12-12	T13		1299.89 ± 28.77	662.73	276.97
25	1998-06-05	F	8.4	2006-11-29	T0	Mother, father	1245.41 ± 13.75	441.4	108.75 ± 18.90
			8.8	2007-04-19	T5		1766.40±2.69	500.34	197.20 ± 31.62
			9.3	2007-10-10	T11		944.99 ± 25.37	476.76	115.66 ± 10.09
25	2001-06-04	M	5.4	2006-11-29	T0	Mother, father	1181.70 ± 50.65	303.75	157.81 ± 11.99
			5.8	2007-04-19	T5		1707.51 ± 30.62	319.63	113.24 ± 2.45
			6.3	2007-10-10	T11		867.79 ± 25.36	364.76	114.76 ± 33.42
26	1994-03-18	F	12.7	2006-11-29	T0	Mother	676.95 ± 9.57	432.08	86.09
27	1987-12-13	F	19	2006-12-19	T0	Father	287.27 ± 8.96	572.38	101.88 ± 13.89
28	2003-05-23	F	3.6	2006-12-19	T0	Mother	612.92 ± 3.03	760.08	45.57 ± 3.40
29	1990-10-17	M	16.2	2006-12-19	T0	Mother	459.54 ± 29.16	488.33	99.03 ± 54.21
			17.0	2007-10-10	T10		505.24 ± 39.04	441.73	121.53 ±15.54
29 (652)	1999-05-11	F	7.6	2006-12-19	T0	Mother	576.64 ± 20.73	656.77	114.39
			8.4	2007-10-10	T10		972.66 ± 7.97	636.32	138.53±16.69
29 (160)	1996-12-02	F	10.0	2006-12-19	T0	Mother	583.62 ± 19.18	600.16	136.79±10.66
			10.8	2007-10-10	T10		874.79 ± 2.17	535.48	112.73±7.74
30	1995-03-09	M	11.8	2006-12-19	T0	Mother	1608.98 ± 8.37	607.15	115.19 ± 6.27
			12.3	2007-07-04	T7		1107.95 ± 0.53	504.15	40.04 ± 11.63
			12.8	2008-01-23	T13		1578.17 ± 18.50	469.62	93.33 ± 3.68
30	1997-06-08	F	9.5	2006-12-19	T0	Mother	1211.80 ± 5.47	586.43	172.18 ± 4.00
			10.1	2007-07-04	T7		774.18 ± 21.15	534.59	40.03 ± 11.95
			10.6	2008-01-23	T13		697.49 ± 12.25	473.45	95.89 ± 6.16
31	1998-03-18	F	8.8	2006-12-19	T0	Mother, aunt, grand-father	467.80 ± 1.39	574.23	106.48 ± 29.19
31	1999-11-03	M	7.1	2006-12-19	T0	Mother, aunt, grand-father	745.53 ± 40.56	552.66	98.22 ± 1.18
32	2004-06-20	F	2.5	2006-12-19	T0	Mother, grand-mother	1573.79 ± 0.72	576.5	142.70 ± 0.57
			3.1	2007-07-04	T7		1034.97 ± 25.55	494.82	52.38 ± 5.01
			3.6	2008-01-23	T13		1237.94 ± 48.60	374.2	152.27 ± 0.32
33	1996-05-17	M	10.7	2007-01-10	T0	Mother	623.78 ± 2.66	649.44	166.16 ± 32.22
			11.5	2007-11-07	T10		671.14 ± 0.27	634.5	36.87 ± 2.05
33	1996-06-25	F	11.2	2007-01-10	T0	Mother	893.13 ± 34.21	436.86	92.74 ± 2.45

			11.7	2007-07-11	T6		716.31 ± 27.52	543.59	37.95 ± 5.33
34	1996-08-14	F	10.3	2006-12-21	T0	Mother	1135.80 ± 18.20	508.95	256.64 ± 37.18
			10.8	2007-06-13	T6		594.41 ± 0.37	490.61	96.56 ± 2.45
			11.4	2008-01-23	T13		978.10 ± 49.46	450.46	103.67 ± 10.95
34	1994-06-21	M	12.5	2006-12-21	T0	Mother	1010.70 ± 22.34	416.71	172.33 ± 50.68
			13.0	2007-06-13	T6		739.31 ± 3.43	499.04	93.55 ± 6.90
			13.6	2008-01-23	T13		777.22 ± 39.78	448.93	92.70 ± 21.91
35 (605)	1995-03-31	M	11.8	2006-12-21	T0	Mother	1126.22 ± 46.08	552.37	163.66 ± 0.79
35 (604)	1995-03-31	M	11.8	2006-12-21	T0	Mother	933.16 ± 14.20	437.43	118.57 ± 6.65
35	1993-05-12	F	13.6	2006-12-21	T0	Mother	1679.45	436.58	128.45 ± 17.60
36	1998-09-06	M	8.3	2007-01-10	T0	Mother	1520.81 ± 20.48	485.39	225.68 ± 85.59
			9.2	2007-11-14	T10		1103.50 ± 27.07	899.87	114.96 ± 0.11
37	2001-07-11	F	5.5	2007-01-17	T0	Mother	419.51±10.21	524.02	35.52 ± 0.52
			6.0	2007-07-04	T6		606.10 ± 14.32	490.91	209.23
38	1995-01-19	M	12.0	2007-01-17	T0	Mother	435.87 ± 7.38	600.34	164.49 ± 10.01
38	1992-08-02	F	14.4	2007-01-17	T0	Mother	328.67 ± 25.67	564.58	166.19 ± 2.53
39	1996-06-08	M	10.6	2007-01-24	T0	Mother	437.90 ± 23.91	529.14	215.53 ± 70.15
			11.1	2007-07-18	T6		617.26 ± 5.45	445.15	146.08 ±8.82
39	1997-08-08	F	9.4	2007-01-24	T0	Mother	399.82 ± 14.71	452.38	71.339 ± 22.51
			9.9	2007-07-18	T6		648.28 ± 6.30	462.01	188.78±12.79
40	1996-05-05	F	10.9	2007-04-05	T0	Mother	986.26 ± 9.88	478.27	99.9
40	1999-04-23	M	8.0	2007-04-05	T0	Mother	851.99 ± 4.04	710.05	52.81 ± 12.17
41	1995-03-29	F	12.2	2007-05-30	T0	Father	500.68 ± 20.08	416.56	71.27 ± 0.30
42	1996-07-03	M	10.8	2007-05-02	T0	Father	391.38 ± 30.03	620.65	32.83
			11.3	2007-11-14	T6		393.23 ± 4.22	445.78	167.25 ±27.97
42	1992-04-14	F	15.1	2007-05-02	T0	Father	452.43 ± 1.68	519.81	38.46 ± 16.02
			15.6	2007-11-14	T6		658.95 ± 1.62	938.89	232.91± 2.00
43	2001-11-20	F	5.5	2007-05-23	T0	Mother	892.70 ± 21.23	484.89	97.65 ± 30.81
44	1995-09-11	M	11.8	2007-06-13	T0	Mother	1058.59 ± 6.11	547.8	41.15 ± 11.08
			12.2	2007-12-12	T6		1160.10 ± 16.16	456.22	145.61 ± 51.30
45	1994-05-10	F	13.2	2007-08-29	T0	Mother	714.66 ± 6.88	482.12	120.00 ± 13.64
			13.8	2008-02-13	T6		801.53 ± 42.46	358.64	134,84 ± 16,18
46	1999-11-04	M	7.8	2007-09-12	T0	Mother	603.75 ± 10.96	569.62	111.95 ± 5.86
46 (980)	1996-04-15	F	11.4	2007-09-13	T0	Mother	504.38 ± 35.85	540.29	118.25 ± 9.11

46 (982)	2004-01-24	F	3.7	2007-09-12	T0	Mother	718.72 ± 78.98	510.97	153.13 ± 4.50
47	1996-12-07	F	10.8	2007-10-17	T0	Mother	1010.10 ± 17.02	494.12	147.00±87.36
47	1999-04-03	M	8.5	2007-10-17	T0	Mother	844.83 ± 30.84	456.7	156.33±50.36
C6	1997-02-06	F	10.3	2007-05-22	T0	Mother	669.60 ± 4.19	755.65	133.68 ± 4.10
			11.0	2008-01-16	T8		733.30 ± 11.16	620.67	250.52 ± 38.11
C15	1997-05-27	M	10.0	2007-06-06	T0	Brother	441.81 ± 0.64	640.33	106.53 ± 1.88
			10.5	2007-12-04	T6		444.69 ± 3.82	958.24	151.86 ± 17.41

* Plus–minus values are means ± standard deviations.

† All subjects are examined before sample collection by an orthopedic surgeon to monitor possible scoliosis development.

EXAMPLE 11

OPN, sCD44 and HA levels in non AIS scoliotic patients

[00158] OPN levels were measured in non AIS scoliotic patients (NAIS patients). Results are summarized in Table 9 below. A comparison of OPN, sCD44 and HA levels in healthy, AIS and NAIS patients is also provided in Figure 12.

Table 9. Biomarkers Comparison of non-AIS scoliotic Patients.

Type of Scoliosis	Characteristics					
	Number	Mean Age (Years)	Mean Cobb Angle	Mean OPN Concentration (ng/ml)	Mean sCD44 Concentration (ng/ml)	Mean HA Concentration (ng/ml)
Neurological Scoliosis	8	12.3 ± 3.7	79.4 ± 15.1	982 ± 452	274 ± 196	127 ± 101
Congenital Scoliosis	8	10.0 ± 4.4	51.8 ± 18.1	1016 ± 400	432 ± 79	123 ± 80
Spondylolisthesis	5	17.5 ± 2.1	21.0 ± 17.0	832 ± 125	386 ± 193	76 ± 54
Kyphosis Scoliosis	5	14.4 ± 2.8	80.2 ± 28.5	923 ± 393	352 ± 62	91 ± 56
Other *	2	15.1	74.5 ± 17.7	586 ± 52	240	NA

† Plus–minus values are means ± standard deviations
* Other scoliosis types include one neuromuscular scoliosis and one dysplasic scoliosis.

[00159] Table 10 below presents in detail biomarkers levels for non AIS scoliotic patients.

Table 10. Clinical and biochemical profiles of non AIS scoliotic patients.

Patient ID	Date of Birth	Gender	Age	Collection Date	Diagnosis	Cobb's Angle Pre-op	Curve Type	Date of Surgery	Age at Surgery	Family History	[OPN] (ng/ml)	[sCD44] (ng/ml)	[HA] (ng/ml)
1208	1990-01-19	M	17.8	2007-10-03	Congenital cyphose scoliosis	72	IT	2004-11-08	14.8	—	1101.06 ± 31.26	444.81	82.89 ± 15.11
1256	1992-03-27	M	13.0	2005-05-09	Congenital scoliosis	44-65	rTIL	2005-03-29	13.0	—	1490.59	NA	127.74 ± 9.29
1278	1998-07-22	F	6.8	2005-05-30	Congenital neurological scoliosis	60	IT	2005-05-30	6.8	—	1401.88	NA	75.65 ± 5.16
1281	1985-05-21	M	20.1	2005-06-01	Spondylolisthesis	16	—	2005-06-01	20.1	—	985.85	NA	150.30 ± 7.93
1286	1990-05-08	M	15.1	2005-06-15	Dysplasic scoliosis	62-66	rTIL	2005-06-15	15.1	—	549.60 ± 5.06	NA	NA
1356	1993-02-22	F	13.2	2006-04-03	Congenital scoliosis	75	rT	2006-04-03	13.2	—	1181.85	NA	111.51 ± 2.30
1358	2003-11-09	M	2.4	2006-04-04	Congenital scoliosis	33-35	rTIL	2006-04-04	2.4	—	1530.6	NA	284.60 ± 69.00
1367	1993-12-12	F	12.4	2006-02-01	Neurological scoliosis	90	ITL	2006-05-01	12.4	—	1525.13	NA	350.01 ± 36.55
1368	1990-06-21	F	15.9	2006-05-02	Neurological cyphosis	50	ITL	2006-05-02	15.9	—	1079.23	NA	126.44 ± 3.63
1370	1995-09-15	M	10.7	2006-05-09	Neurological scoliosis	65	rT	2006-05-09	10.7	—	1318.58	NA	104.06 ± 5.18
1375	1992-09-13	F	13.7	2006-05-30	Congenital scoliosis	53	rTL	2006-05-30	13.7	Cousin	380.08 ± 12.95	NA	NA
1407	1990-12-22	M	16.8	2007-10-31	Spondylolisthesis	9	IL	2006-09-25	15.8	—	818.17 ± 1.52	441.73	116.09 ± 3.88
1431	1987-11-23	M	19.2	2007-01-08	Neurological scoliosis	90-90	rTIT	2007-01-08	19.2	—	450.78 ± 101.56	275.62	130.30 ± 23.92
1432	1992-08-08	M	14.4	2007-01-09	Neurological scoliosis	64	rT	2007-01-09	14.4	—	558.47 ± 4.70	145.15	98.99 ± 13.92
1434	1994-08-07	F	12.4	2007-01-10	Congenital scoliosis	79-77	rTIL	2007-01-10	12.4	—	631.59 ± 7.42	325.95	44.79 ± 5.73
1436	1993-02-16	F	13.9	2007-01-22	Cyphose scoliosis	120	—	2007-01-22	13.9	—	220.32± 2.94	322.03	44.34 ± 6.37
1437	1992-11-06	M	14.2	2007-02-05	Neurological scoliosis	100	NA	2007-02-05	14.2	—	388.01 ± 8.22	225.71	76.96 ± 4.53
1455	1996-12-14	F	10.3	2007-04-03	Congenital cyphose scoliosis	61	ITL	2007-04-03	10.3	—	1090.51 ± 5.57	323.24	34.79 ± 0.32
1456	1990-10-03	F	16.5	2007-04-17	Neuromuscular scoliosis	87	rTL	2007-04-17	16.5	—	622.46 ± 7.15	240.22	NA
1462	1997-10-22	F	9.5	2007-04-23	Neurological scoliosis	76	ITL	2007-04-23	9.5	—	1118.25 ± 1.32	607.1	55.90 ± 1.82
1463	1989-03-19	F	18.1	2007-04-24	Scoliosis+Spondylolisthesis	33	rT	2007-04-	18.1	—	751.54 ± 8.69	284.71	21.56 ± 4.58

1466	1997-08-24	F	9.8	2007-05-08	Congenital scoliosis	39	rL	2007-05-08	9.8	—	1110.01 ± 2.38	510.18	47.07 ± 1.48
1475	1993-05-25	M	14.1	2007-06-05	Cyphose scoliosis	98	—	2007-06-04	14.1	—	1123.49 ± 5.56	319.93	166.63 ± 34.63
1479	1996-01-24	F	11.4	2007-06-05	Neurological scoliosis	90	rTIL	2007-06-05	11.4	—	1098.54 ± 131.44	119.17	NA
1480	2003-06-13	F	4.0	2007-06-18	Congenital scoliosis	56	lT	2007-06-18	4.0	—	809.8	468.03	120.72 ± 40.73
1482	1989-03-30	F	18.2	2007-06-19	Spondylolisthesis gr 1	—	NA	2007-06-19	18.2	—	678.49 ± 18.32	187.48	46.07 ± 5.27
1486	1993-01-15	M	14.4	2007-06-27	Spondylolisthesis gr 2	—	NA	2007-06-27	14.4	—	924.40 ± 17.16	628.78	47.06 ± 6.84
357	1996-07-08	F	11.4	2007-12-18	Congenital scoliosis	30-31	rTlT	—	—	—	996.58 ± 8.51	423.72	127.33 ± 3.13

* Plus–minus values are means ± standard deviations.

† Curve type nomenclature: r, right/ l, left/ T, Thoracic/ L, Lumbar/ TL, Thoracolumbar/ C, Cervical

EXAMPLE 12

OPN and sCD44 levels in AIS patients pre and post operations

[00160] OPN levels were measured in AIS patients pre (n=79) and post (N=28) operations. Interestingly, comparison of AIS patients in pre-operation vs. post operation showed a reduction in circulating OPN levels, which further support the role of OPN at the cellular level as mechanosensor (Figure 13).

[00161] OPN were measured in AIS female patients pre (n=10) and post (N=10) treatment with braces. Similarly, sCD44 levels were measured in AIS female patients pre (n=15) and post (N=12) operations. Results are presented in Figure 14.

[00162] A distribution of 12 AIS patients was also performed across the predefined cut-off zones pre-operation and post-operation. Figure 15 shows 92% of the surgically treated patients had pre-operation OPN levels in the red-zone (>800ng/mL of plasma OPN level), while the remaining 8% were in the yellow zone (700-800ng/mL). No patients were in the green zone representing plasma OPN levels <700 ng/mL. This also shows a strong correlation between high OPN concentrations and the progression of scoliotic curves.

[00163] Panel B of Figure 15 show that red zone patients who were treated surgically experienced a decline in OPN concentrations in the blood. 75% of the surgically treated patients fell into the green and yellow zones (800 ng/mL or less).

EXAMPLE 13

OPN levels in AIS patients with various types of braces

[00164] OPN levels were also measured in AIS patients prior to being treated with brace (n=79) and after brace (N=28). Table 11 below also shows the effect of braces on biomarkers.

Table 11. Possible effects of brace treatment on biomarker concentrations.

Treatment	Characteristics						
	No.	Mean Age (Years)	Mean Brace Wear (Months)	Mean Cobb's Angle	Mean OPN Concentration (ng/ml)	Mean sCD44 Concentration (ng/ml)	Mean HA Concentration (ng/ml)

Without Brace							
Female	193	14.2 ± 2.1	-	30.9 ± 19.3	809 ± 376	474 ± 179	108 ± 58
Male	36	14.8 ± 2.2	-	32.2 ± 21.1	1034 ± 376	492 ± 155	126 ± 62
With Brace (All Female)							
All Braces Combined	21	14.0 ± 1.8	12.0	21.2 ± 8.3	664 ± 282	483 ± 112	118 ± 60
Boston	5	13.0 ± 1.4	10.6	25.8 ± 4.4	735 ± 358	568 ± 184	150 ± 57
SpineCor	14	14.5 ± 1.6	12.7	20.6 ± 8.7	626 ± 279	451 ± 81	108 ± 62
Charleston	1	15.4	10.0	7.0	781	532	70
Providence Night Brace	1	9.7	1.0	20.0	732	547	138
P-value ‡					0.018	0.879	0.608

* Plus–minus values are means ± standard deviations.
‡ Statistical analysis to compare patients with or without brace was done by bilateral unpaired Student's T-test with equal variance. A difference was considered statistically significant with a p-value < 0.05.

[00165] A distribution of AIS patients across the predefined cut-off zones was also performed prior to being treated with bracing and after bracing. Eight patients were tested a certain number of months after bracing, namely for each of patients #1 to 8: 7, 7, 8, 22, 22, 22 and 26 months after bracing, respectively. Figure 16 shows that prior to being treated with bracing (Panel A), 63% of these patients were in the red and yellow zones. A significant shift towards the green zone (<700ng/mL) was observed, which is consistent with the trend observed in surgically treated patients, as presented in Figures 13 -15.

EXAMPLE 14

Comparison of selenium levels in AIS patients vs. healthy subjects

[00166] Selenium concentration was reported to be significantly decreased in plasma of AIS patients (42). Selenium and more specifically Se-methylselenocystein, an

organoselenium naturally occurring in diet, are used to prevent metastasis in breast cancer as chemopreventive therapy by targeting OPN transcription (43-45).

[00167] Plasma selenium concentration was thus measured in pediatric populations (AIS vs. healthy controls) to determine whether or not low selenium levels correlate with higher OPN concentrations in AIS. Plasma selenium concentrations were determined by a fluorometric method using 2,3-diaminonaphthalene (DAN) (46, 47). Results presented in Figures 18 and 19 show a correlation between high OPN levels and low selenium levels in scoliotic and asymptomatic at risk children.

[00168] Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

REFERENCES

(1) Brodner W, Krepler P, Nicolakis M et al. Melatonin and adolescent idiopathic scoliosis. J Bone Joint Surg Br 2000; 82(3):399-403.

(2) Lowe TG, Edgar M, Margulies JY et al. Etiology of idiopathic scoliosis: current trends in research. J Bone Joint Surg Am 2000; 82-A(8):1157-1168.

(3) Veldhuizen AG, Wever DJ, Webb PJ. The aetiology of idiopathic scoliosis: biomechanical and neuromuscular factors. Eur Spine J 2000; 9(3):178-184.

(4) Miller NH. Cause and natural history of adolescent idiopathic scoliosis. Orthop Clin North Am 1999; 30(3):343-52, vii.

(5) Miller NH. Genetics of familial idiopathic scoliosis. Clin Orthop 2002;(401):60-64.

(6) Miller NH, Schwab DL, Sponseller PD, Manolio TA, Pugh EW, Wilson AP. Characterization of idiopathic scoliosis in a clinically well-defined population. Clin Orthop 2001;(392):349-357.

(7) Wise CA, Barnes R, Gillum J, Herring JA, Bowcock AM, Lovett M. Localization of susceptibility to familial idiopathic scoliosis. Spine 2000; 25(18):2372-2380.

(8) Moreau A, Wang DS, Forget S et al. Melatonin Signaling Dysfunction in Adolescent Idiopathic Scoliosis. Spine 2004.

(9) Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest 2001; 107(9):1055-1061.

(10) Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J. Osteopontin--a molecule for all seasons. QJM 2002; 95(1):3-13.

(11) Lopez CA, Olson ES, Adams JC, Mou K, Denhardt DT, Davis RL. Osteopontin expression detected in adult cochleae and inner ear fluids. Hear Res 1995; 85(1-2):210-222.

(12) Simoneau M, Richer N, Mercier P, Allard P, Teasdale N. Sensory deprivation and balance control in idiopathic scoliosis adolescent. Exp Brain Res 2006; 170(4):576-582.

(13) Guo X, Chau WW, Hui-Chan CW, Cheung CS, Tsang WW, Cheng JC. Balance control in adolescents with idiopathic scoliosis and disturbed somatosensory function. Spine 2006; 31(14):E437-E440.

(14) Weber B, Rosel M, Arch R, Moller P, Zoller M. Transient expression of CD44 variant isoforms in the ontogeny of the rat: ectoderm-, endoderm- and mesoderm-derived cells express different exon combinations. Differentiation 1996; 60(1):17-29.

(15) Panda D, Kundu GC, Lee BI et al. Potential roles of osteopontin and alphaVbeta3 integrin in the development of coronary artery restenosis after angioplasty. Proc Natl Acad Sci U S A 1997; 94(17):9308-9313.

(16) Ruiz P, Schwarzler C, Gunthert U. CD44 isoforms during differentiation and development. Bioessays 1995; 17(1):17-24.

(17) Katagiri YU, Sleeman J, Fujii H et al. CD44 variants but not CD44s cooperate with beta1-containing integrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell motility

and chemotaxis. Cancer Res 1999; 59(1):219-226.

(18) Jalkanen S, Jalkanen M. Lymphocyte CD44 binds the COOH-terminal heparin-binding domain of fibronectin. J Cell Biol 1992; 116(3):817-825.

(19) Naujokas MF, Morin M, Anderson MS, Peterson M, Miller J. The chondroitin sulfate form of invariant chain can enhance stimulation of T cell responses through interaction with CD44. Cell 1993; 74(2):257-268.

(20) Weber GF, Ashkar S, Glimcher MJ, Cantor H. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science 1996; 271(5248):509-512.

(21) Bennett KL, Modrell B, Greenfield B et al. Regulation of CD44 binding to hyaluronan by glycosylation of variably spliced exons. J Cell Biol 1995; 131(6 Pt 1):1623-1633.

(22) Stamenkovic I, Aruffo A, Amiot M, Seed B. The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. EMBO J 1991; 10(2):343-348.

(23) Komura K, Sato S, Fujimoto M, Hasegawa M, Takehara K. Elevated levels of circulating CD44 in patients with systemic sclerosis: association with a milder subset. Rheumatology (Oxford) 2002; 41(10):1149-1154.

(24) Scott DA, Stapleton JA, Palmer RM et al. Plasma concentrations of reputed tumor-associated soluble CD44 isoforms (v5 and v6) in smokers are dose related and decline on smoking cessation. Cancer Epidemiol Biomarkers Prev 2000; 9(11):1211-1214.

(25) Wang X, Jiang H, Raso J et al. Characterization of the scoliosis that develops after pinealectomy in the chicken and comparison with adolescent idiopathic scoliosis in humans. Spine 1997; 22(22):2626-2635.

(26) von Gall C, Lewy A, Schomerus C et al. Transcription factor dynamics and neuroendocrine signalling in the mouse pineal gland: a comparative analysis of melatonin-deficient C57BL mice and melatonin-proficient C3H mice. Eur J Neurosci 2000; 12(3):964-972.

(27) Aherrahrou Z, Axtner SB, Kaczmarek PM et al. A locus on chromosome 7 determines dramatic up-regulation of osteopontin in dystrophic cardiac calcification in mice. Am J Pathol 2004; 164(4):1379-1387.

(28) Machida M, Dubousset J, Yamada T et al. Experimental scoliosis in melatonin-deficient C57BL/6J mice without pinealectomy. J Pineal Res 2006; 41(1):1-7.

(29) Scoliosis Research Society. Morbidity & Mortality Committee annual report 1997.

(30) Mishima R, Takeshima F, Sawai T et al. High plasma osteopontin levels in patients with inflammatory bowel disease. J Clin Gastroenterol 2007; 41(2):167-172.

(31) Ang C, Chambers AF, Tuck AB, Winquist E, Izawa JI. Plasma osteopontin levels are predictive of disease stage in patients with transitional cell carcinoma of the bladder. BJU Int 2005; 96(6):803-805.

(32) Wong CK, Lit LC, Tam LS, Li EK, Lam CW. Elevation of plasma osteopontin concentration is correlated with disease activity in patients with systemic lupus erythematosus. Rheumatology (Oxford) 2005; 44(5):602-606.

(33) Kim J, Ki SS, Lee SD et al. Elevated plasma osteopontin levels in patients with

hepatocellular carcinoma. Am J Gastroenterol 2006; 101(9):2051-2059.

(34) Wynne-Davies R. Familial (idiopathic) scoliosis. A family survey. J Bone Joint Surg Br 1968; 50(1):24-30.

(35) De George FV, Fisher RL. Idiopathic scoliosis: genetic and environmental aspects. J Med Genet 1967; 4(4):251-257.

(36) Lein M, Jung K, Weiss S, Schnorr D, Loening SA. Soluble CD44 variants in the serum of patients with urological malignancies. Oncology 1997; 54(3):226-230.

(37) Karjalainen JM, Tammi RH, Tammi MI et al. Reduced level of CD44 and hyaluronan associated with unfavorable prognosis in clinical stage I cutaneous melanoma. Am J Pathol 2000; 157(3):957-965.

(38) Schlosser W, Gansauge F, Schlosser S, Gansauge S, Beger HG. Low serum levels of CD44, CD44v6, and neopterin indicate immune dysfunction in chronic pancreatitis. Pancreas 2001; 23(4):335-340.

(39) Sjoberg S, Fogelstrand L, Hulthe J, Fagerberg B, Krettek A. Circulating soluble CD44 is higher among women than men and is not associated with cardiovascular risk factors or subclinical atherosclerosis. Metabolism 2005; 54(2):139-141.

(40) Jenkins RH, Thomas GJ, Williams JD, Steadman R. Myofibroblastic differentiation leads to hyaluronan accumulation through reduced hyaluronan turnover. J Biol Chem 2004; 279(40):41453-41460.

(41) Lien YH, Fu J, Rucker RB, Scheck M, Abbott U, Stern R. Collagen, proteoglycan and hyaluronidase activity in cultures from normal and scoliotic chicken fibroblasts. Biochim Biophys Acta 1990; 1034(3):318-325.

(42) Dastych M, Cienciala J. Idiopathic scoliosis and concentrations of zinc, copper, and selenium in blood plasma. Biol Trace Elem Res 2002; 89(2):105-110.

(43) El-Bayoumy K, Sinha R. Molecular chemoprevention by selenium: a genomic approach. Mutat Res 2005; 591(1-2):224-236.

(44) Unni E, Kittrell FS, Singh U, Sinha R. Osteopontin is a potential target gene in mouse mammary cancer chemoprevention by Se-methylselenocysteine. Breast Cancer Res 2004; 6(5):R586-R592.

(45) He YT, Liu DW, Ding LY, Li Q, Xiao YH. Therapeutic effects and molecular mechanisms of anti-fibrosis herbs and selenium on rats with hepatic fibrosis. World J Gastroenterol 2004; 10(5):703-706.

(46) Sheehan TM, Gao M. Simplified fluorometric assay of total selenium in plasma and urine. Clin Chem 1990; 36(12):2124-2126.

(47) Ando M, Takizawa M, Suwabe S, Yamato S, Shimada K. Determination of selenium in human serum by liquid chromatography/electron capture atmospheric pressure chemical ionization mass spectrometry after acid digestion and derivatization using 2,3-diaminonaphthalene. Eur J Mass Spectrom (Chichester, Eng) 2003; 9(6):619-622.

(48) Uchio E, Matsuura N, Kadonosono K, Ohno S, Uede T. Tear osteopontin levels in patients with allergic conjunctival diseases. Graefes Arch Clin Exp Ophthalmol, 2002; 240(11): 924-8.

(49) Buck et al. Design Strategies and Performance of Custom DNA Sequencing

primers . Biotechniques 1999; 27:528-536.

(50) Ponta, H, Sherman L, Herrlich, PA. CD44 : from Adhesion molecules to signalling regulators. Nature Reviews. 2004; 4:33-45.

(51) Garrett, K.A., P.D. Esker, and A.H. Sparks. 2007. Introduction to the R Programming Environment. The Plant Health Instructor. DOI:10.1094/PHI-A-2007-1226-02.

(52) Ihaka R, Gentleman R. A language for data analysis and graphics. Journal of Computational and Graphical Statistics1996, 5(3):299-314.

(53) Goodison S, and Tarin D. Clinical Implications Of Anomalous Cd44 Gene Expression In Neoplasia. Frontiers in Bioscience 1998, 3, e89-109.

(54) Ito T, Hashimoto Y, Tanaka E, Kan T, Tsunoda S, Sato F, Higashiyama M, Okumura T, Shimada Y. An Inducible Short-Hairpin RNAVector against Osteopontin Reduces Metastatic Potential of Human Esophageal Squamous Cell Carcinoma In vitro and In vivo Clin Cancer Res 2006;12(4) 1308-1316.

(55) Kadkol SS, Lin AY, Barak V, Kalickman I, Leach L, Valyi-Nagy K, Majumdar D, Setty S, Maniotis A J, Folberg R, Pe'er J. Osteopontin Expression and Serum Levels in Metastatic Uveal Melanoma — A Pilot Study Invest Ophthalmol Vis Sci. 2006; 47(3): 802–806.

(56) Guarino V, Faviana P, Salvatore G, Castellone MD, Cirafici A , De Falco V, Celetti A, Giannini R, Basolo F, Melillo RM, Santoro M. Osteopontin Is Overexpressed in Human Papillary Thyroid Carcinomas and Enhances Thyroid Carcinoma Cell Invasiveness. The Journal of Clinical Endocrinology & Metabolism.2005 90(9):5270–5278.

(57) Ponta et al, Nat Rev Mol Cell Biol. 2003 Jan;4(1):33-45. Review.

CLAIMS:

1. A method for determining the risk for developing a scoliosis comprising monitoring osteopontin (OPN) expression in a sample from a subject over time;
wherein an OPN expression that increases in the subject sample over time is indicative that the subject is at risk for developing a scoliosis.

2. The method of claim 1, wherein the monitoring begins when the subject is about three years old.

3. The method of claim 1, wherein the monitoring is performed by measuring OPN expression at a frequency of at least about once per month.

4. The method of claim 1, wherein the monitoring is performed by measuring OPN expression at a frequency of at least about once per six month.

5. The method of any one of claims 1 to 4, wherein the monitoring OPN expression is performed using an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).

6. A method for determining the risk for developing a scoliosis comprising measuring osteopontin (OPN) expression in a sample from a subject;
wherein an OPN expression that is higher in the subject sample than that in a control sample is indicative that the subject is at risk for developing a scoliosis.

7. The method of any one of claims 1 to 6, wherein the subject is a likely candidate for developing a scoliosis.

8. The method of any one of claims 1 to 7, wherein the subject is a likely candidate for developing adolescent idiopathic scoliosis.

9. The method of any one of claims 1 to 8, wherein the subject is pre-diagnosed as having a scoliosis.

10. The method of any one of claims 1 to 9, wherein the subject is pre-diagnosed

with adolescent idiopathic scoliosis.

11. A method of stratifying a subject having a scoliosis comprising

measuring osteopontin (OPN) expression in a sample from the subject;

whereby the measuring step enables the stratification of the subject into a scoliosis subgroup.

12. A method for assessing the efficacy of a brace on a subject having a scoliosis comprising

measuring osteopontin (OPN) expression in a sample from the subject prior to and at least once after bracing the subject,

wherein an increase in the OPN expression after as compared to prior to bracing the subject is indicative that the brace is ineffective.

13. The method of claim 11, wherein the determining the OPN expression after the bracing is performed at least one month after the bracing.

14. The method of claim 11, wherein the determining the OPN expression after bracing the subject is performed at least 2 months hours after the bracing.

15. The method of claim 12, wherein the determining the OPN expression after bracing the subject is performed at least three months after the bracing.

16. The method of claim 12, wherein the determining the OPN expression after bracing the subject is performed at least six months after the bracing.

17. The method of any one of claims 1 to 16, wherein the method further comprises measuring soluble CD44 receptor (sCD44) expression in the sample from the subject.

18. The method of any one of claims 1 to 17, wherein the sample from the subject is a biological fluid from the subject.

19. The method of claim 18, wherein the biological fluid is selected from the group consisting of blood, urine, tear and saliva.

20. The method of claim 19, wherein the biological fluid is plasma.

21. The method of any one of claim 1 to 20, wherein the OPN expression is OPN protein.

22. The method of claim 21, wherein the determining of the OPN expression is performed with an antibody that specifically binds to OPN.

23. The method of claim 22, wherein the measuring OPN expression is performed using an enzyme-linked immunosorbent assay (ELISA).

24. The method of claim 23, wherein the sample is a plasma sample and an OPN expression that is higher than 700 nanograms per milliliter of plasma is indicative that the subject is at risk for developing a scoliosis.

25. The method of claim 23, wherein the sample is a plasma sample and an OPN expression that is higher than 800 nanograms per milliliter of plasma is indicative that the subject is at risk for developing a scoliosis.

26. The method of any one of claim 1 to 20, wherein the OPN expression is OPN RNA.

27. The method of any one of claim 1 to 17, wherein the sample from the subject is a paraspinal muscle biopsy and the OPN expression is OPN RNA.

28. A method of selecting an agent as a potential candidate for the reduction or prevention of scoliosis comprising contacting a candidate agent with a cell expressing osteopontin (OPN), and detecting the expression of OPN, wherein when the expression of OPN is lower in the presence of the candidate agent as compared to in the absence thereof, the candidate agent is selected.

29. A method of selecting an agent as a potential candidate for the reduction or prevention of scoliosis comprising contacting a candidate agent with a cell expressing sCD44, and detecting the expression of sCD44, wherein when the expression of OPN is higher in the presence of the candidate agent as compared to in the absence thereof,

the candidate agent is selected.

30. The method of any one of claims 28 and 29, wherein the cell is a cell derived from a scoliotic patient.

31. A method of selecting an agent as a potential candidate for the prevention or reduction of scoliosis comprising administering a candidate agent to a scoliosis model animal before scoliosis has developed in the animal, whereby the candidate is selected when the scoliosis is prevented or reduced in the model animal as compared to in a control animal who was not administered the candidate agent.

32. A method of preventing or reducing scoliosis comprising administering to a subject having scoliosis a therapeutically effective amount of an osteopontin inhibitor (OPN) or a selenium rich diet, whereby scoliosis is thereby prevented or treated.

33. A method of preventing or reducing scoliosis comprising administering to a subject having scoliosis a therapeutically effective amount of a CD44 inhibitor, whereby scoliosis is thereby prevented or treated.

34. A method of preventing or reducing scoliosis comprising administering to a subject having scoliosis a therapeutically effective amount of a sCD44 stimulator, whereby scoliosis is thereby prevented or treated.

35. The method of any one of claims 1 to 34 wherein the subject is human.

36. The method of any one of claims 1 to 34 wherein the subject is human female.

37. The method of any one of claims 1 to 34 wherein the subject is human male.

38. An osteopontin inhibitor for use in the treatment or prevention of scoliosis.

39. A CD44 inhibitor for use in the treatment or prevention of scoliosis.

40. A sCD44 stimulator for use in the treatment or prevention of scoliosis.

41. A use of an osteopontin inhibitor in the manufacture of a medicament for the prevention or the treatment of scoliosis.

42. A use of an osteopontin inhibitor for the prevention or the treatment of scoliosis.

43. A use of a CD44 inhibitor in the manufacture of a medicament for the prevention or the treatment of scoliosis.

44. A use of a CD44 inhibitor for the prevention or the treatment of scoliosis.

45. A use of a sCD44 stimulator in the manufacture of a medicament for the prevention or the treatment of scoliosis.

46. A use of a sCD44 stimulator for the prevention or the treatment of scoliosis.

47. The use of any one of claims 41 to 46 wherein the scoliosis is adolescent idiopathic scoliosis.

48. A kit for predicting the risk of developing a scoliosis comprising a ligand specific to osteopontin (OPN) and instructions to use the kit for predicting the risk of developing a scoliosis.

49. The kit of claim 48, further comprising a ligand specific to soluble CD44 (sCD44).

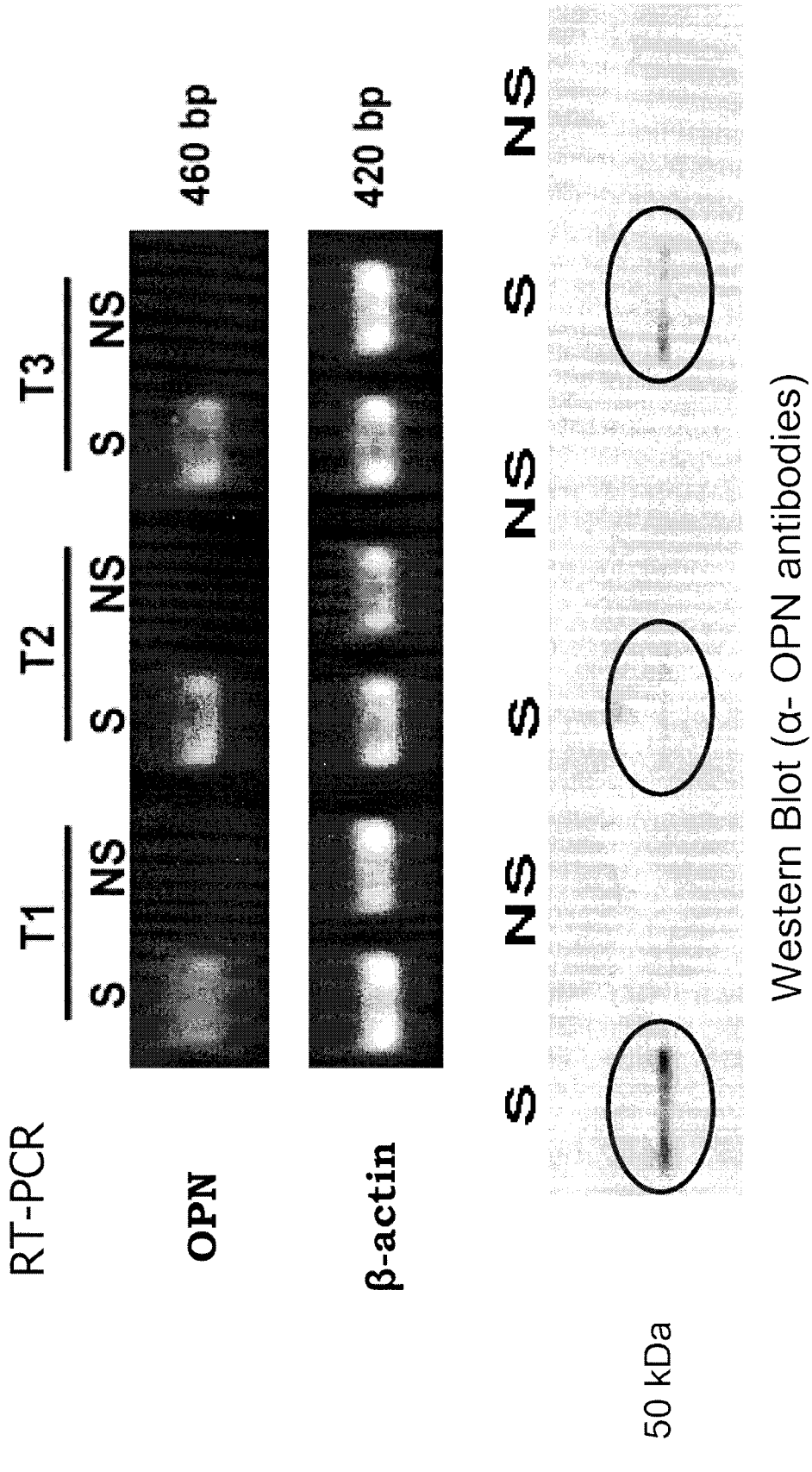
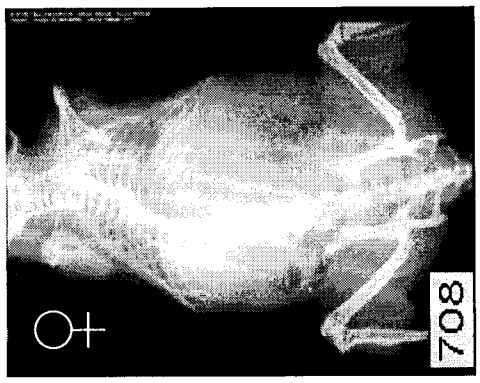
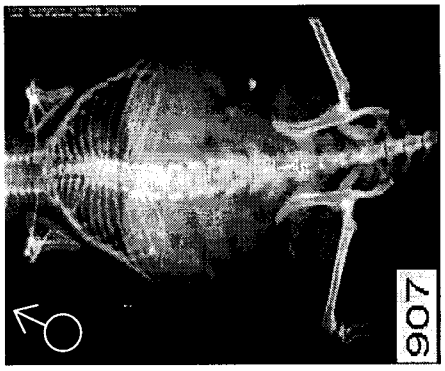

RT-PCR
T1
S
NS
T2
S
NS
T3
S
NS
460 bp
OPN
420 bp
β-actin
S
NS
S
NS
S
NS
50 kDa
Western Blot (α- OPN antibodies)

Figure 1


♀
708


♂
907

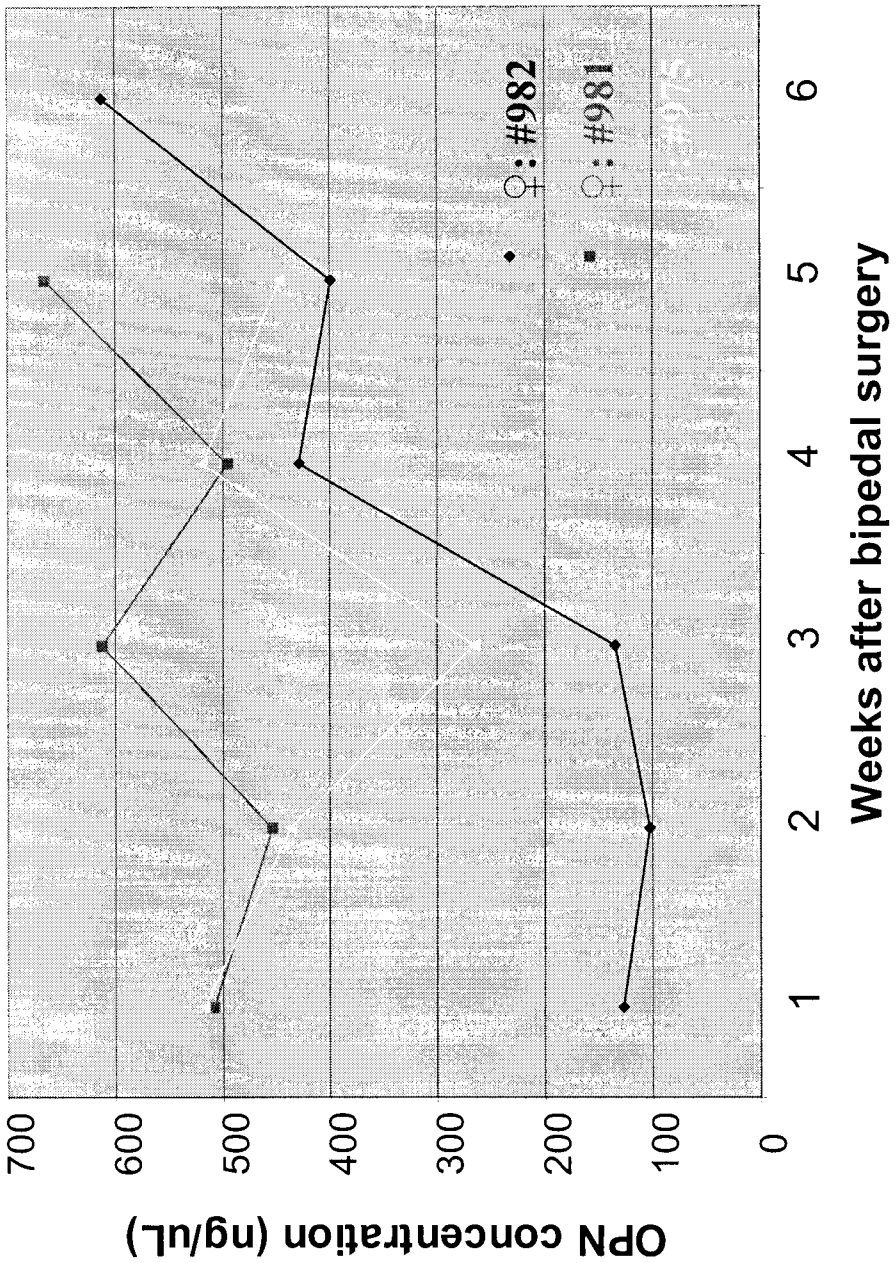
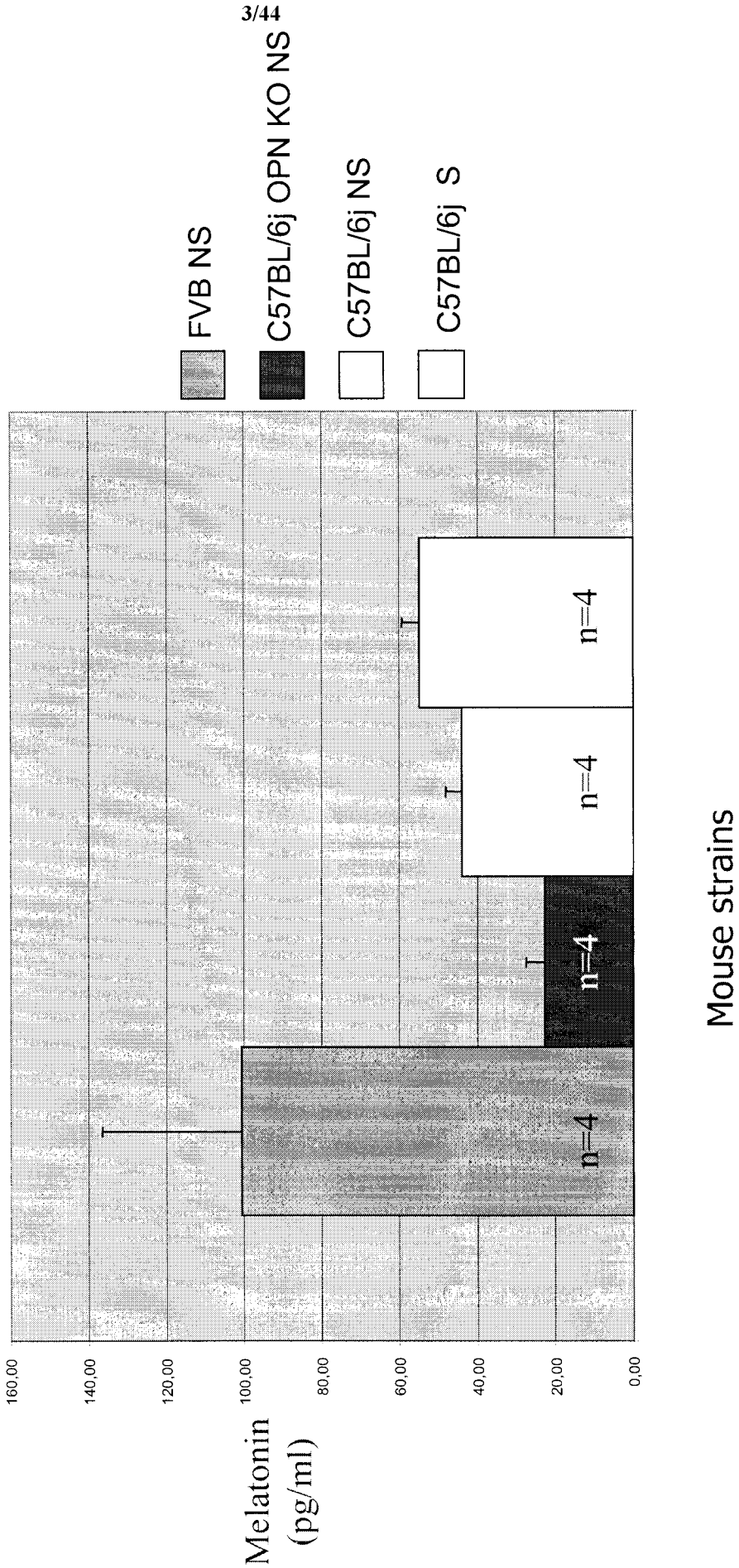

OPN concentration (ng/uL)
0
100
200
300
400
500
600
700
1
2
3
4
5
6
Weeks after bipedal surgery
♀: #982
♀: #981

Figure 2


FVB NS
C57BL/6j OPN KO NS
C57BL/6j NS
C57BL/6j S
160,00
140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Melatonin (pg/ml)
n=4
n=4
n=4
n=4
Mouse strains

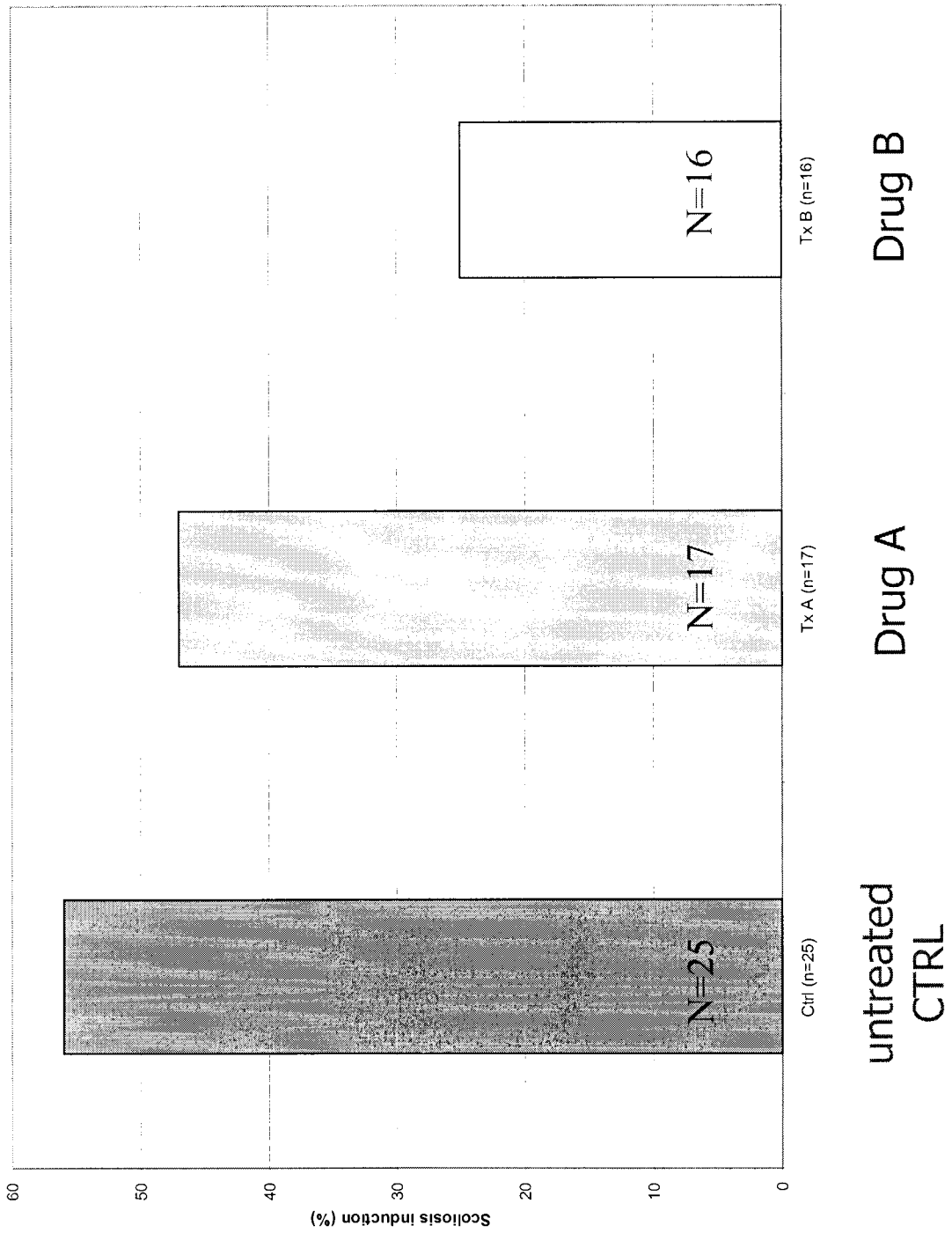

Scoliosis induction (%)
0
10
20
30
40
50
60
N=25
N=17
N=16
Ctrl (n=25)
Tx A (n=17)
Tx B (n=16)
untreated CTRL
Drug A
Drug B

Figure 4

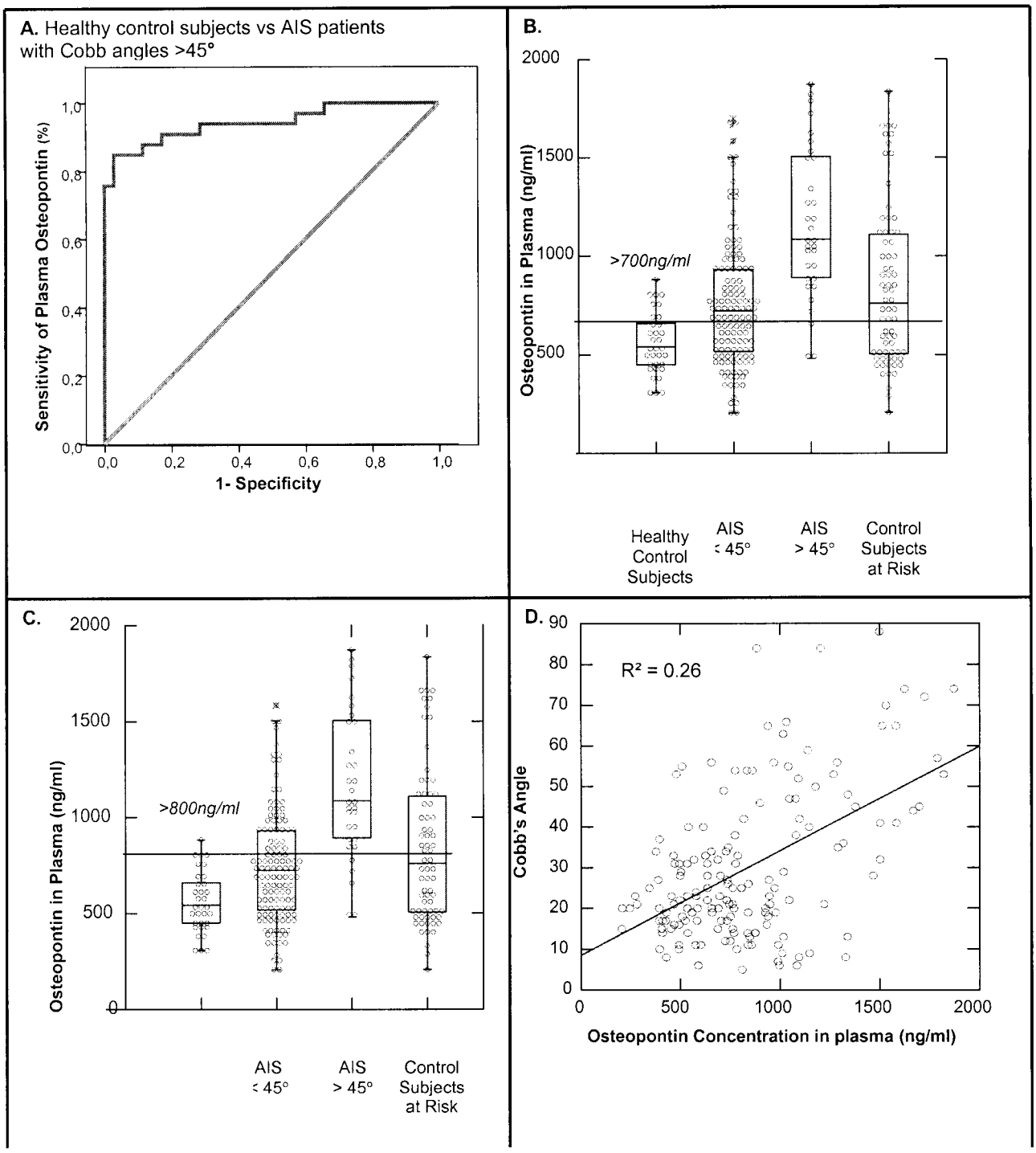
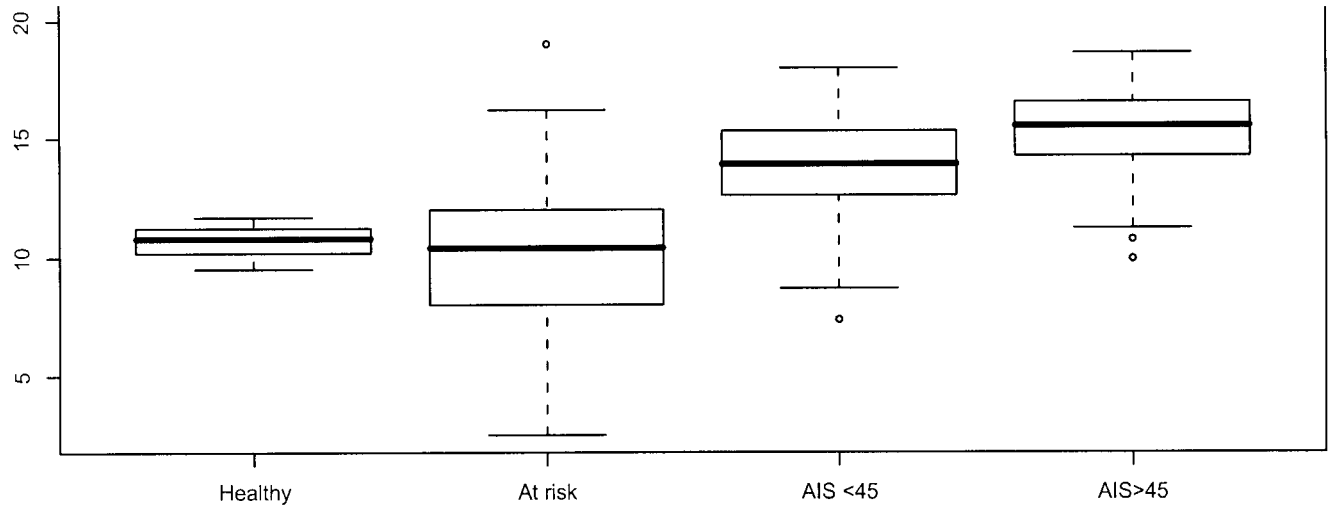
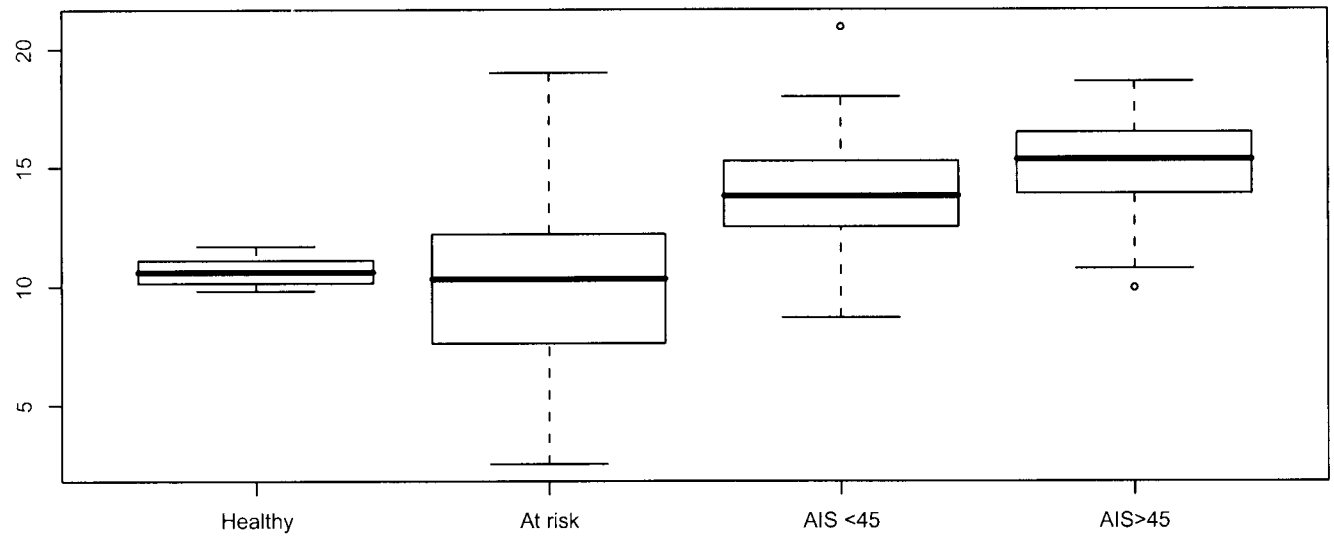

A. Healthy control subjects vs AIS patients with Cobb angles >45°
Sensitivity of Plasma Osteopontin (%)
1- Specificity
B.
Osteopontin in Plasma (ng/ml)
>700ng/ml
Healthy Control Subjects
AIS < 45°
AIS > 45°
Control Subjects at Risk
C.
Osteopontin in Plasma (ng/ml)
>800ng/ml
AIS < 45°
AIS > 45°
Control Subjects at Risk
D.
R² = 0.26
Cobb's Angle
Osteopontin Concentration in plasma (ng/ml)

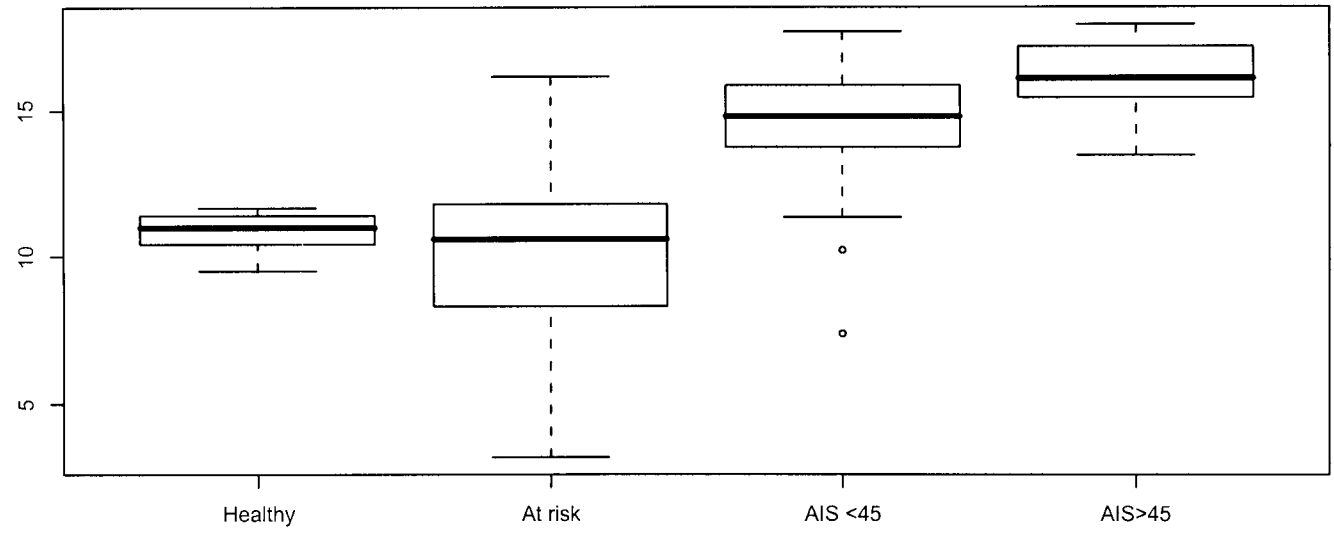
Figure 5



Age girls



Age boys



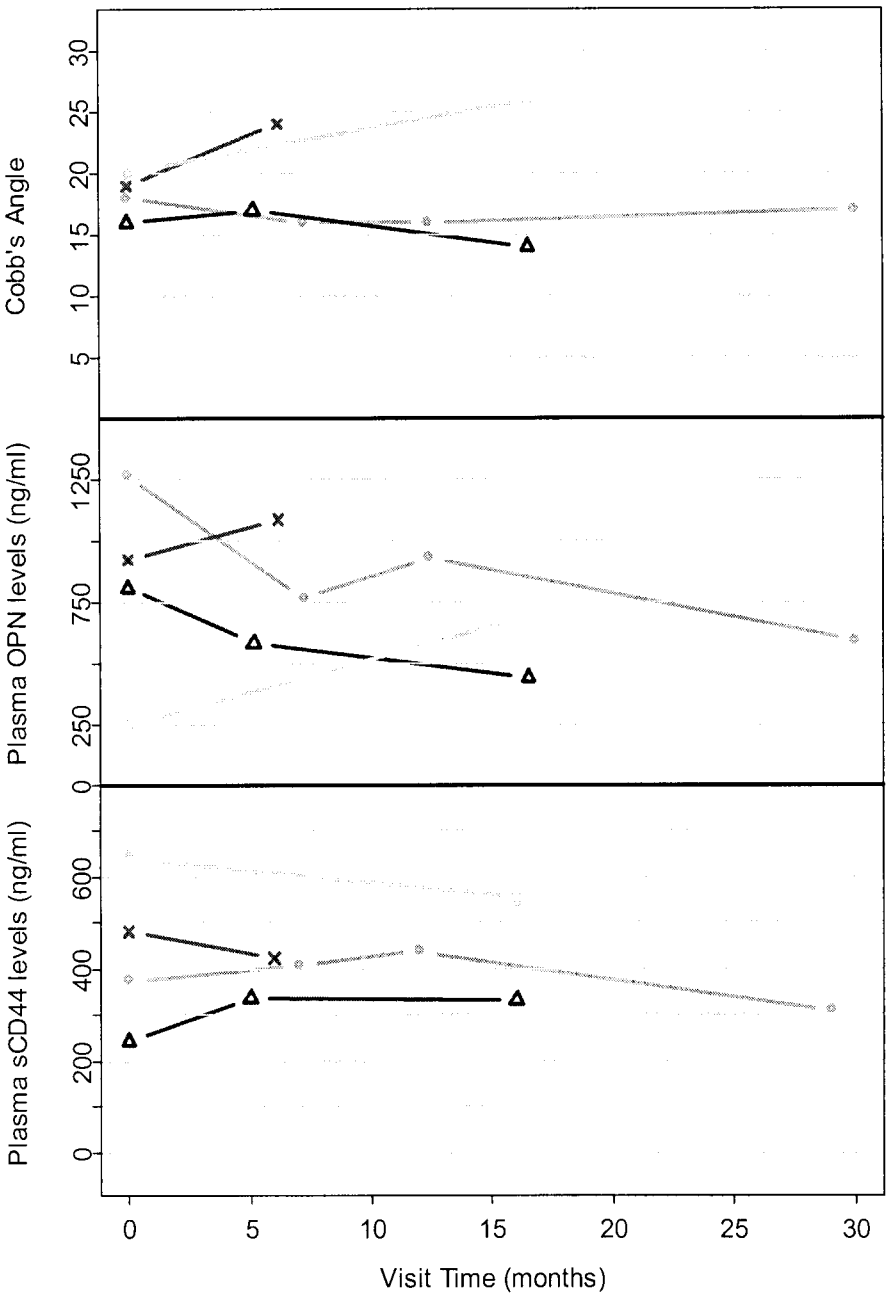

Cobb's Angle
5
10
15
20
25
30
Plasma OPN levels (ng/ml)
0
250
750
1250
Plasma sCD44 levels (ng/ml)
0
200
400
600
0
5
10
15
20
25
30
Visit Time (months)

Figure 7

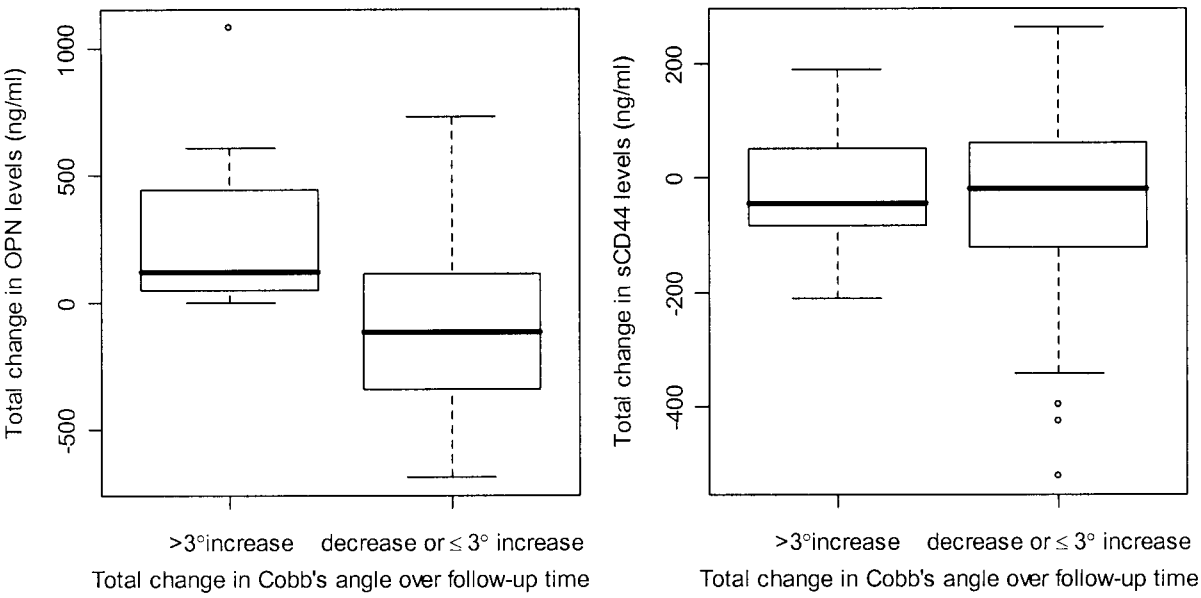
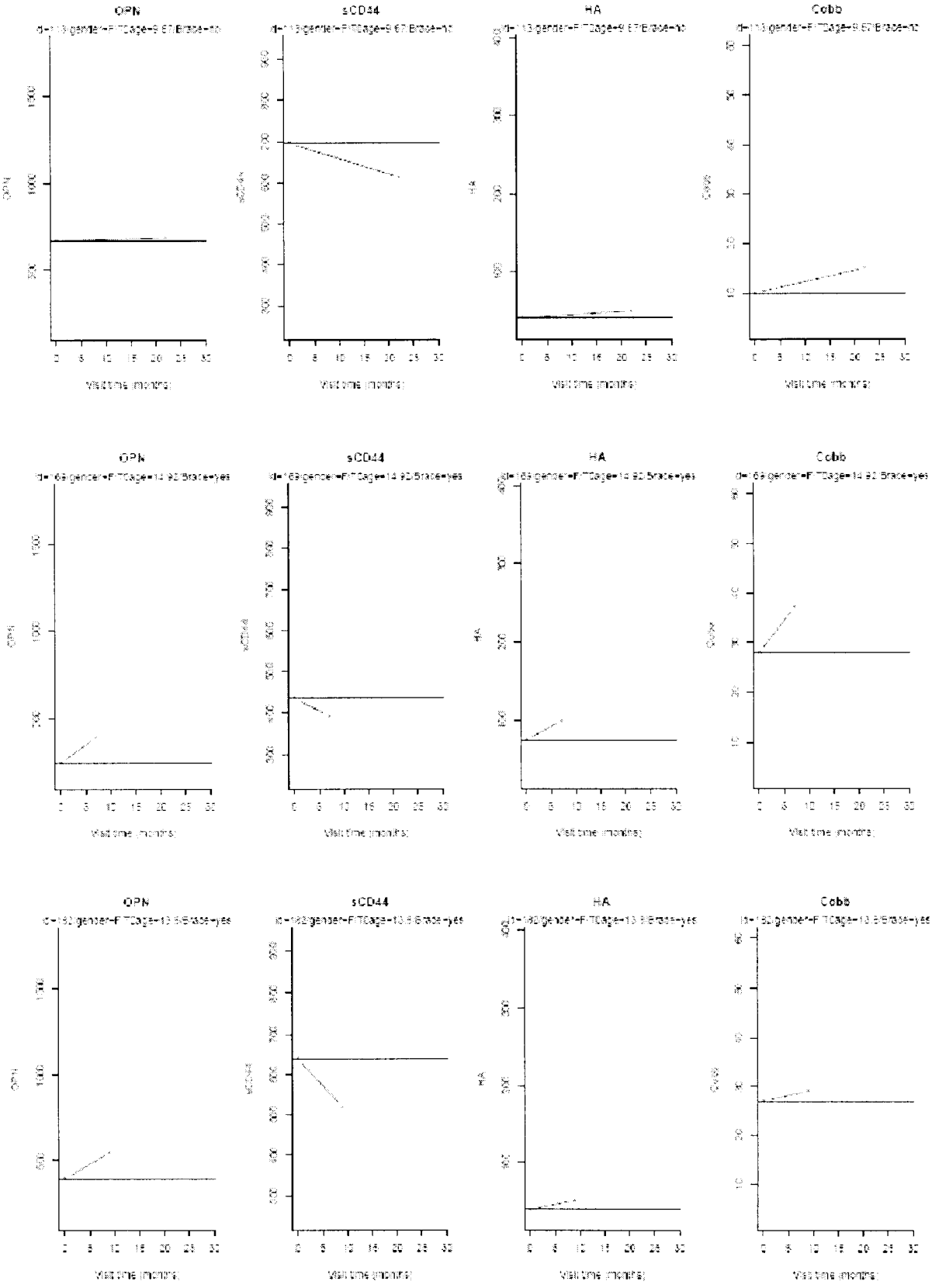
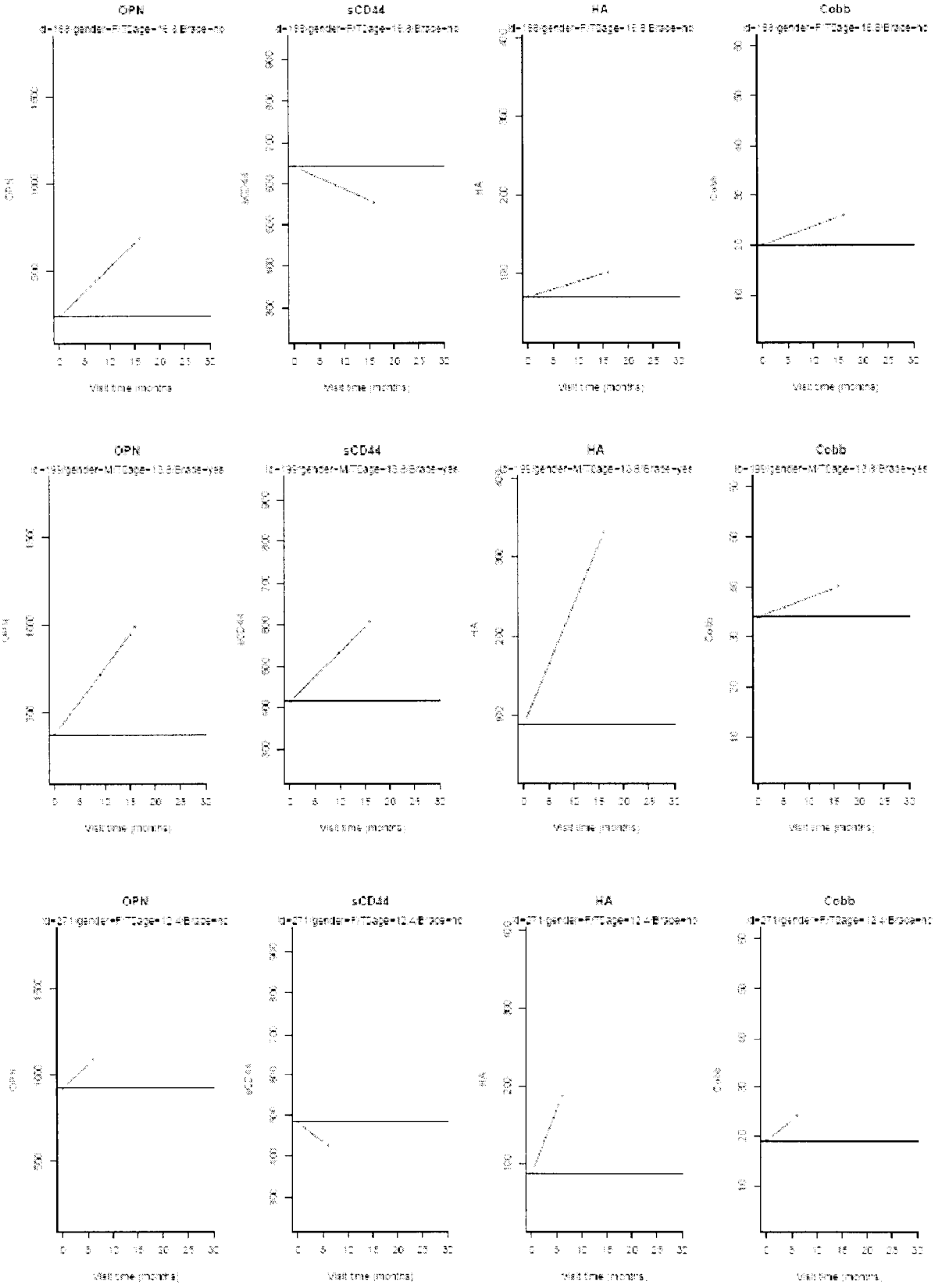
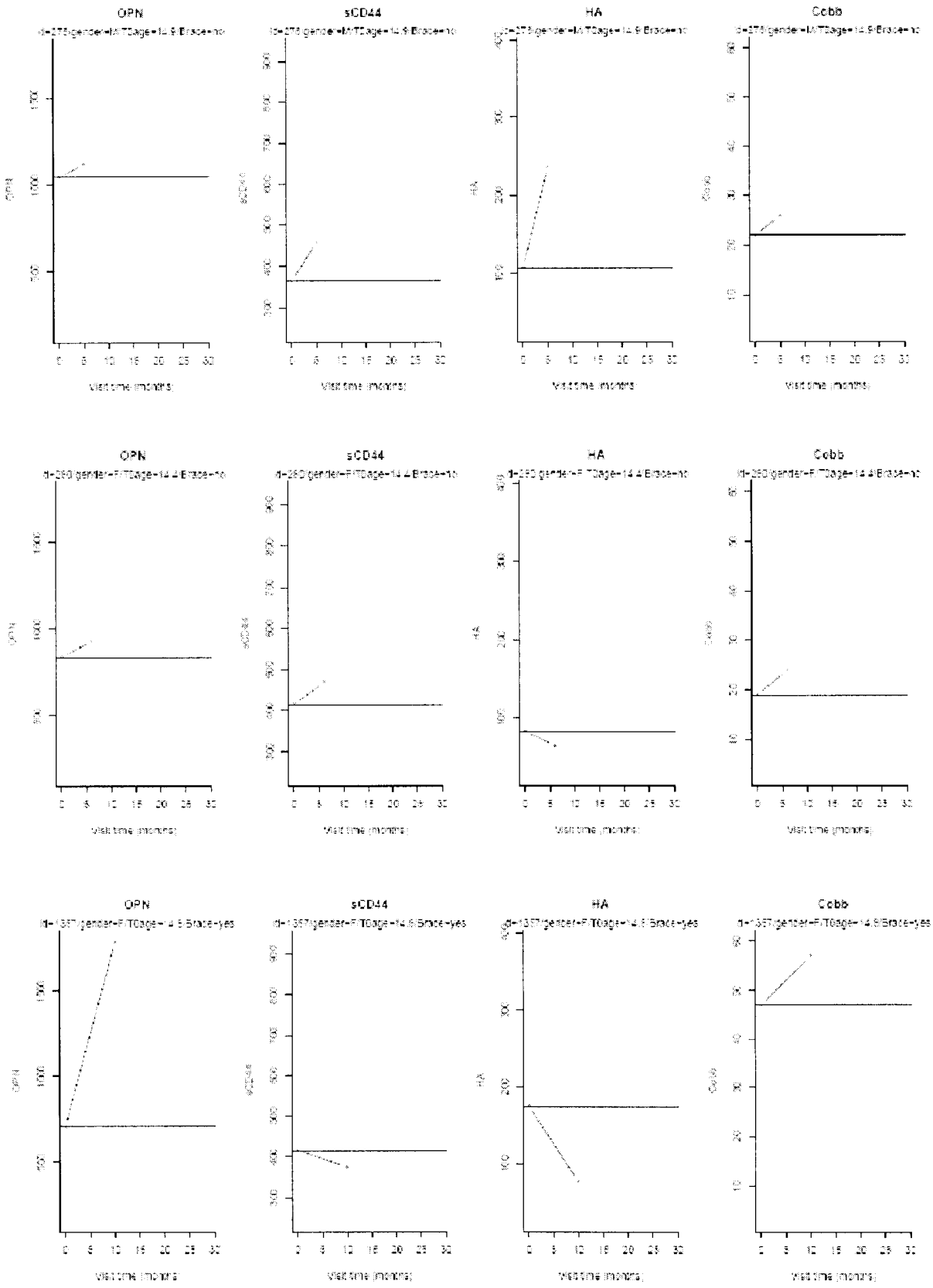
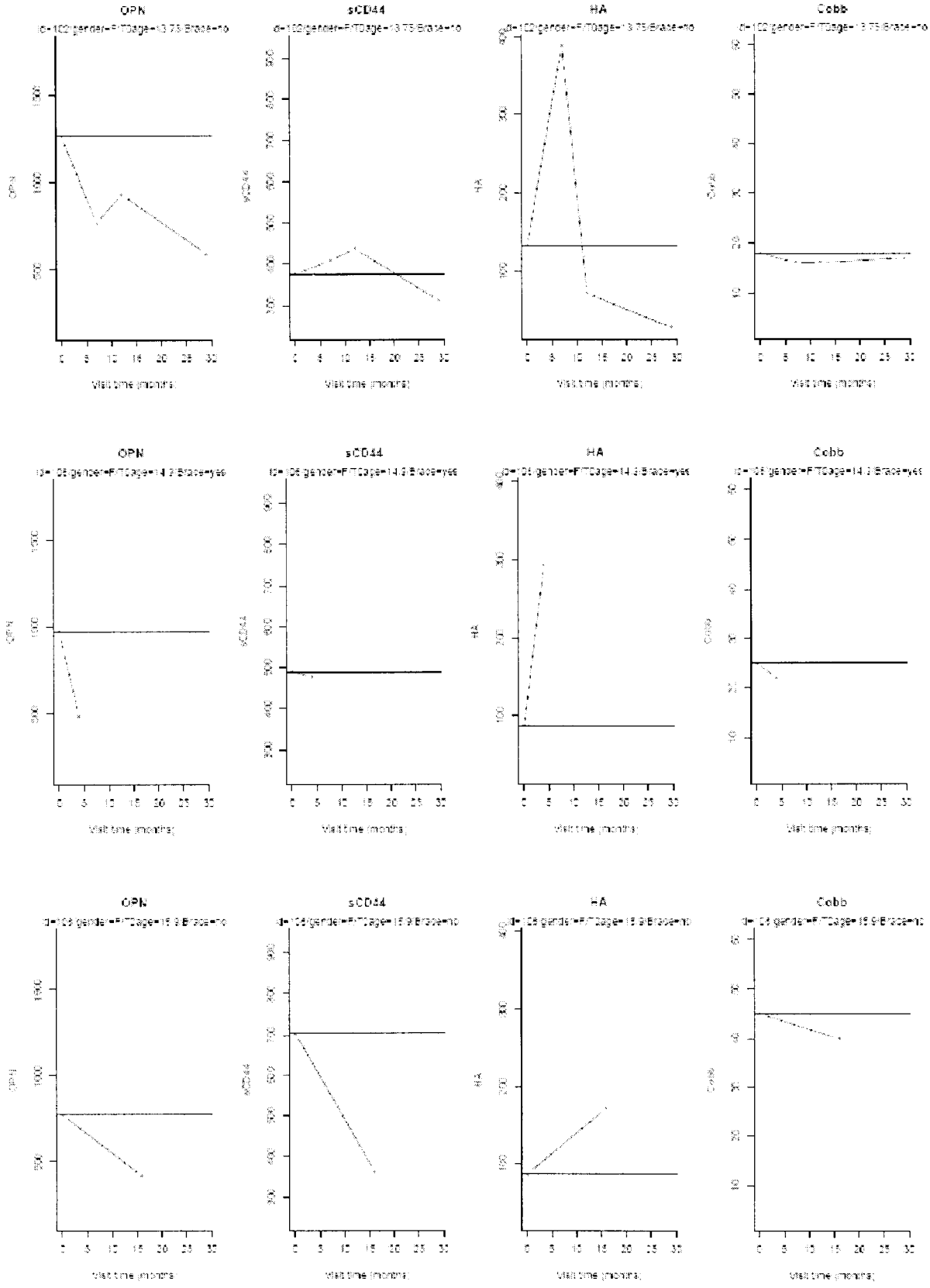

Total change in OPN levels (ng/ml)
-500
0
500
1000
>3°increase
decrease or ≤ 3° increase
Total change in Cobb's angle over follow-up time
Total change in sCD44 levels (ng/ml)
-400
-200
0
200
>3°increase
decrease or ≤ 3° increase
Total change in Cobb's angle over follow-up time

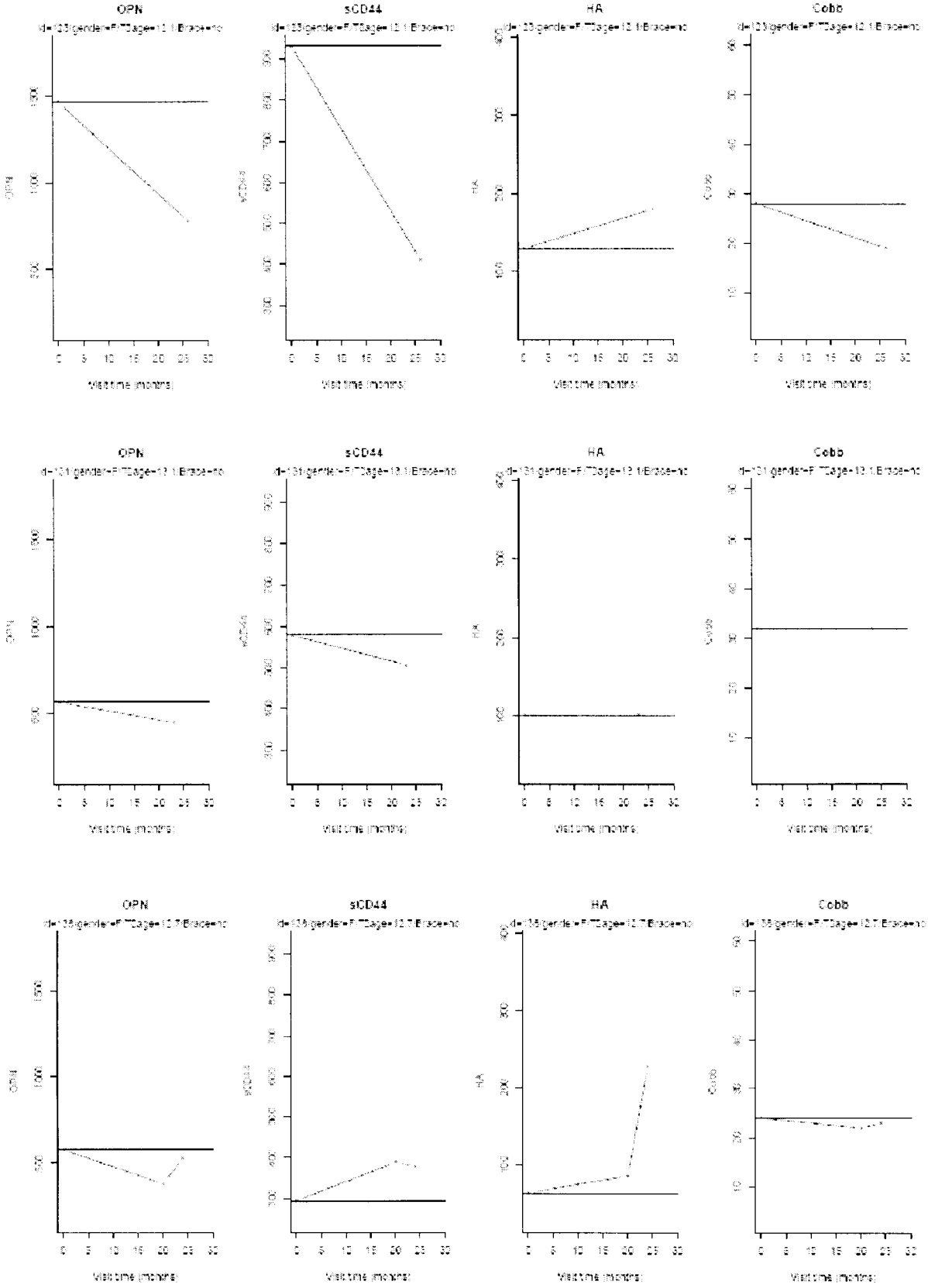
Figure 8

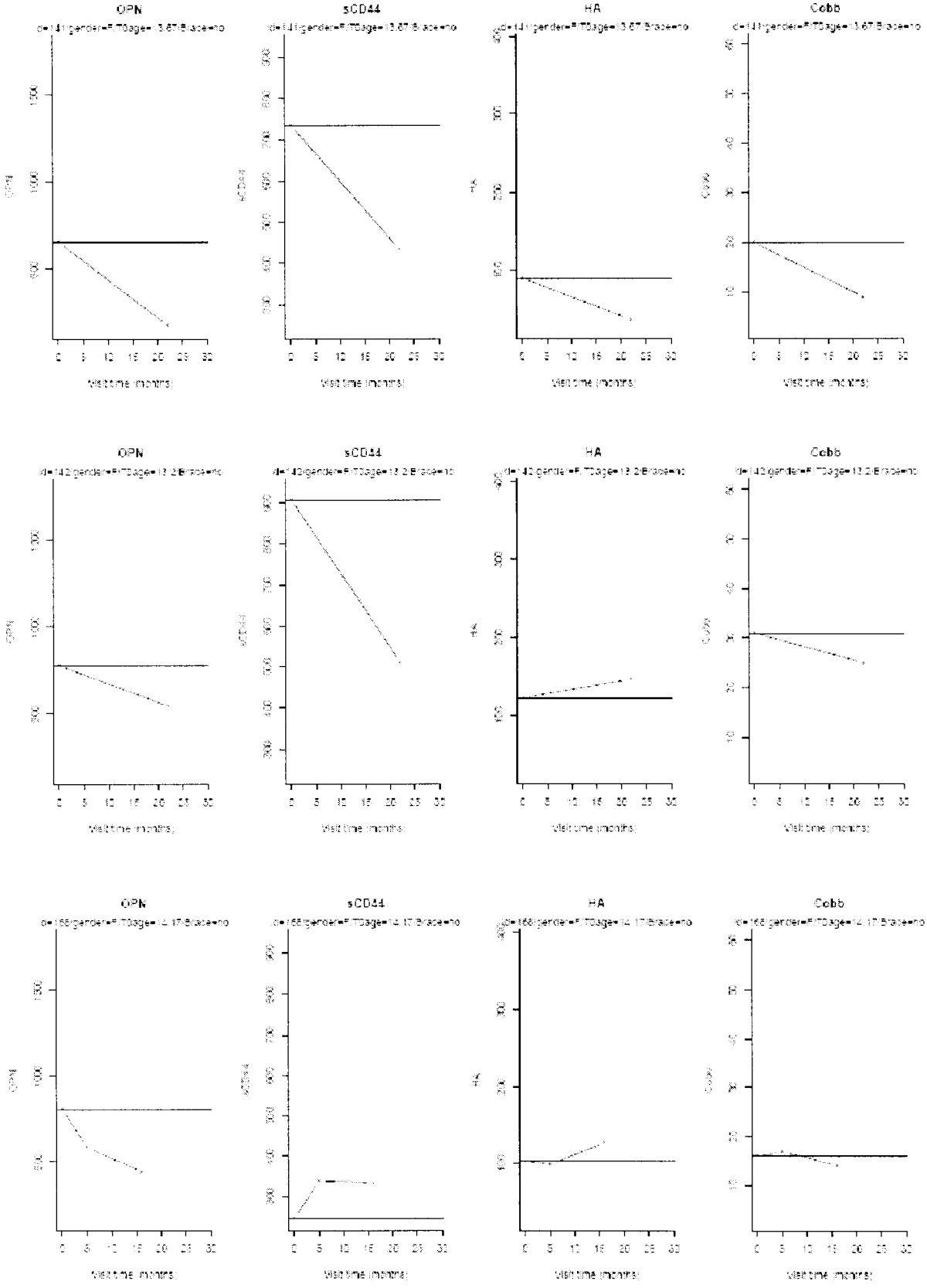

OPN
sCD44
HA
Cobb
Visit time (months)

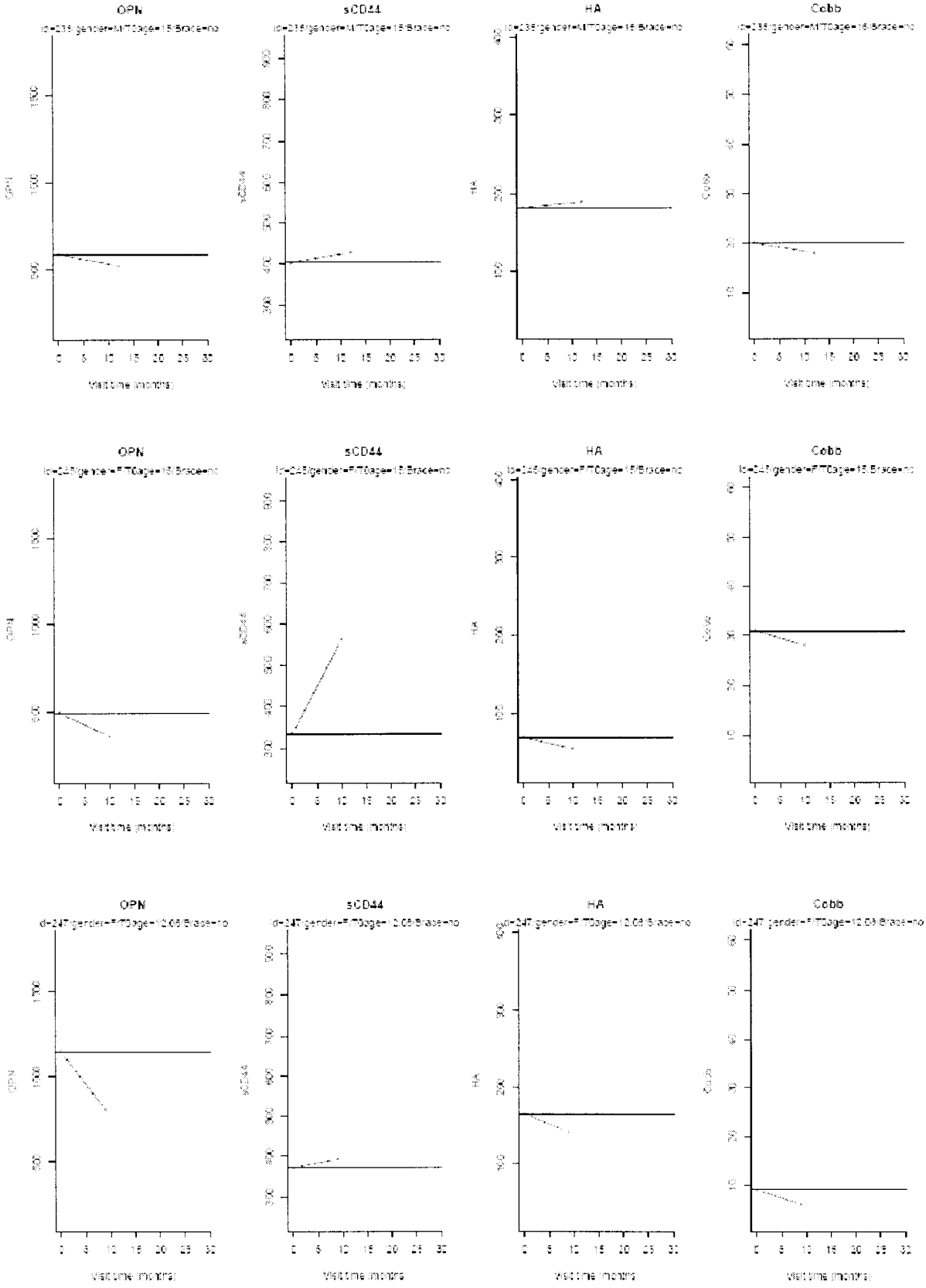

OPN
sCD44
HA
Cobb
Visit time (months)

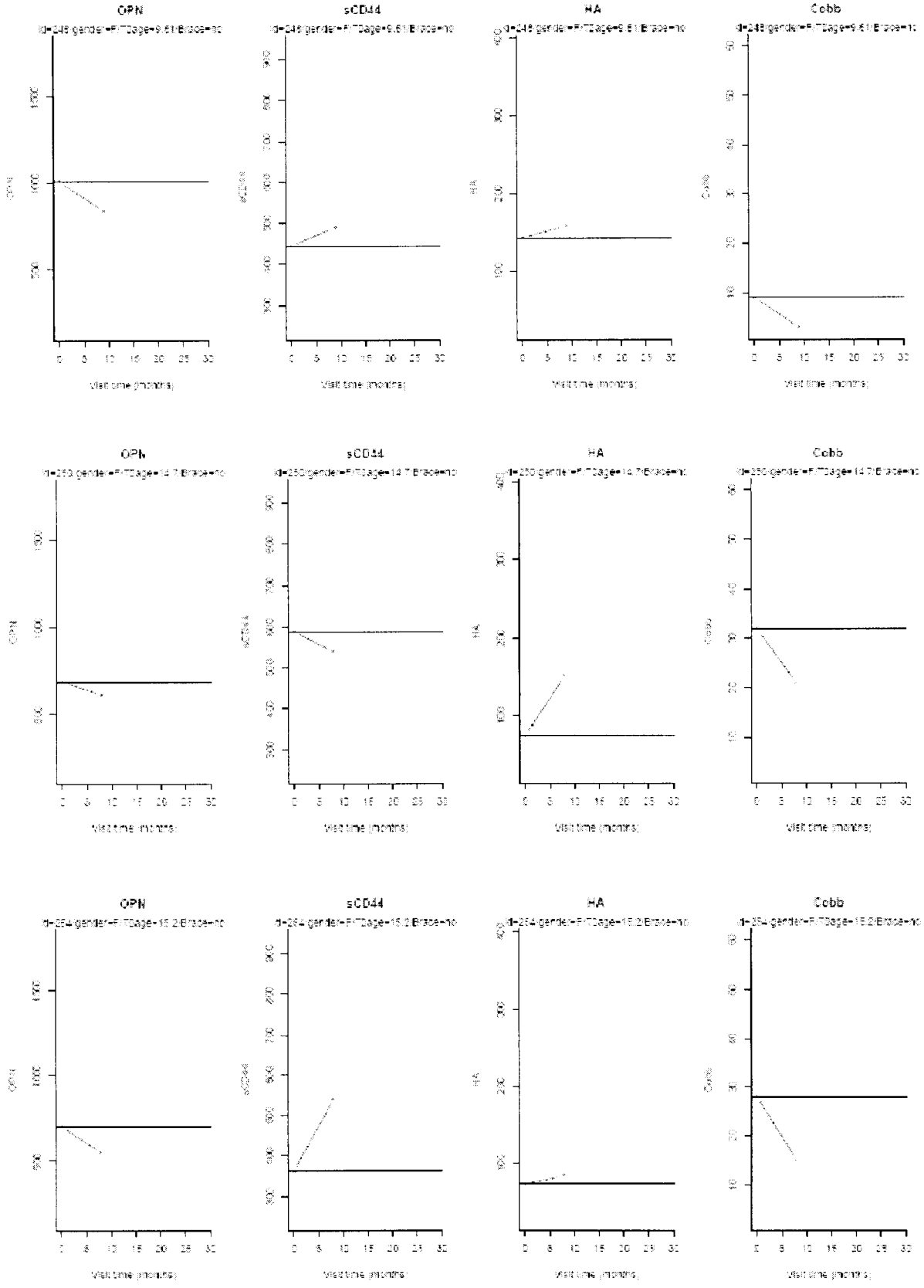

OPN
sCD44
HA
Cobb
Visit time (months)

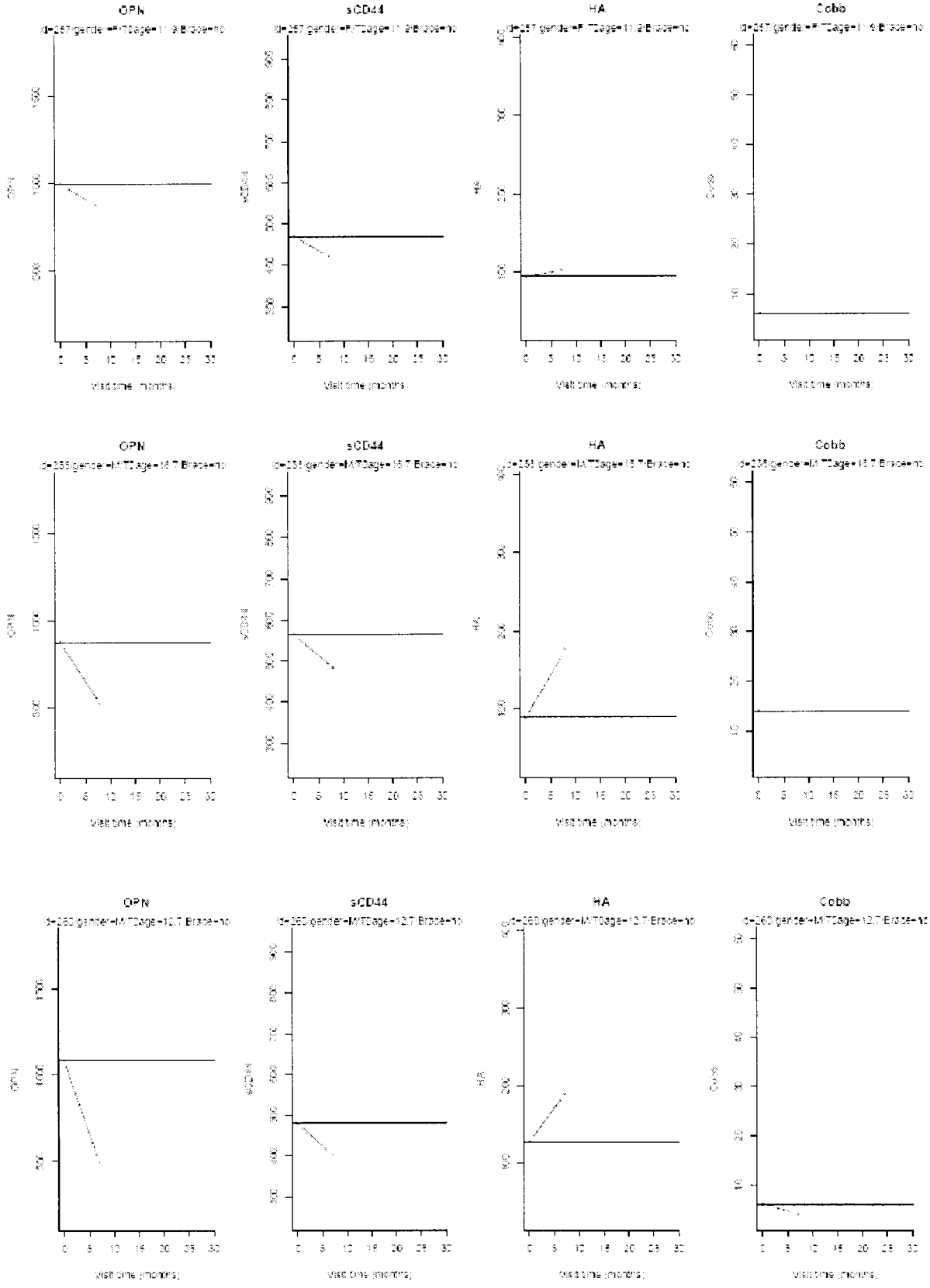

OPN
sCD44
HA
Cobb
Visit time (months)

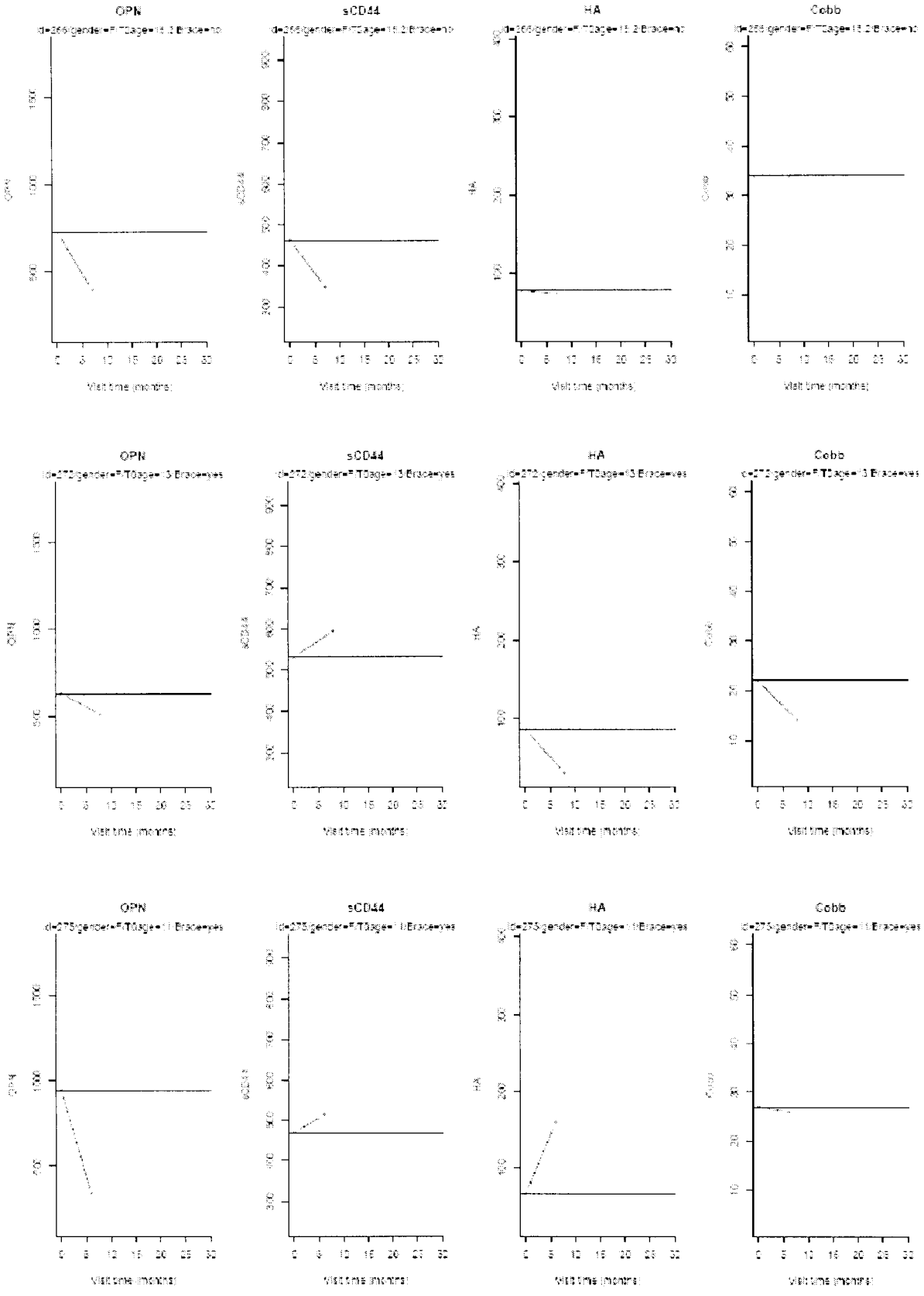

OPN
sCD44
HA
Cobb
Visit time (months)

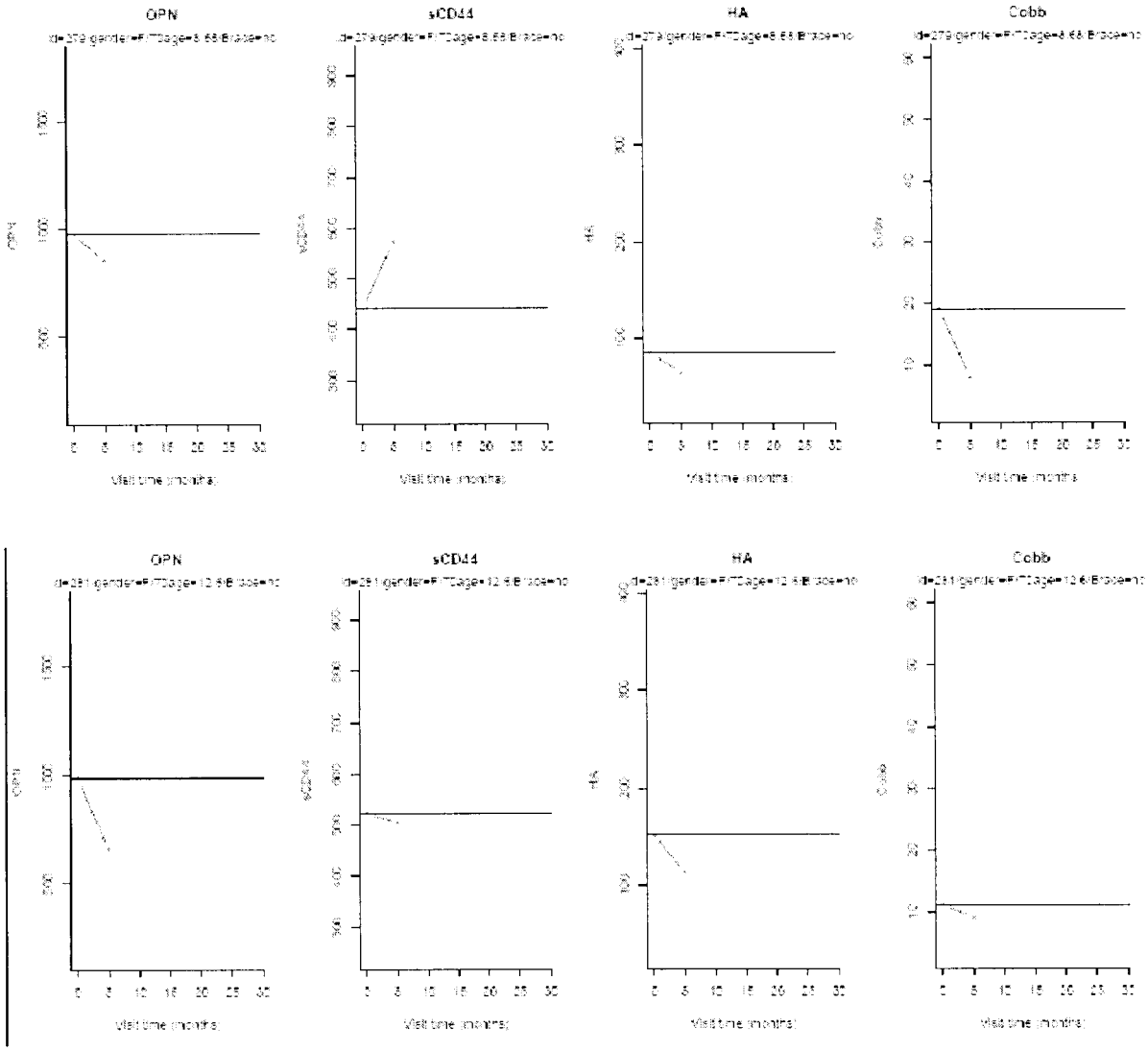

OPN
sCD44
HA
Cobb
Visit time (months)


OPN
sCD44
HA
Cobb
Visit time (months)


OPN
sCD44
HA
Cobb
Visit time (months)


OPN
sCD44
HA
Cobb
Visit time (months)


OPN
sCD44
HA
Cobb
Visit time (months)


OPN
sCD44
HA
Cobb
Visit time (months)

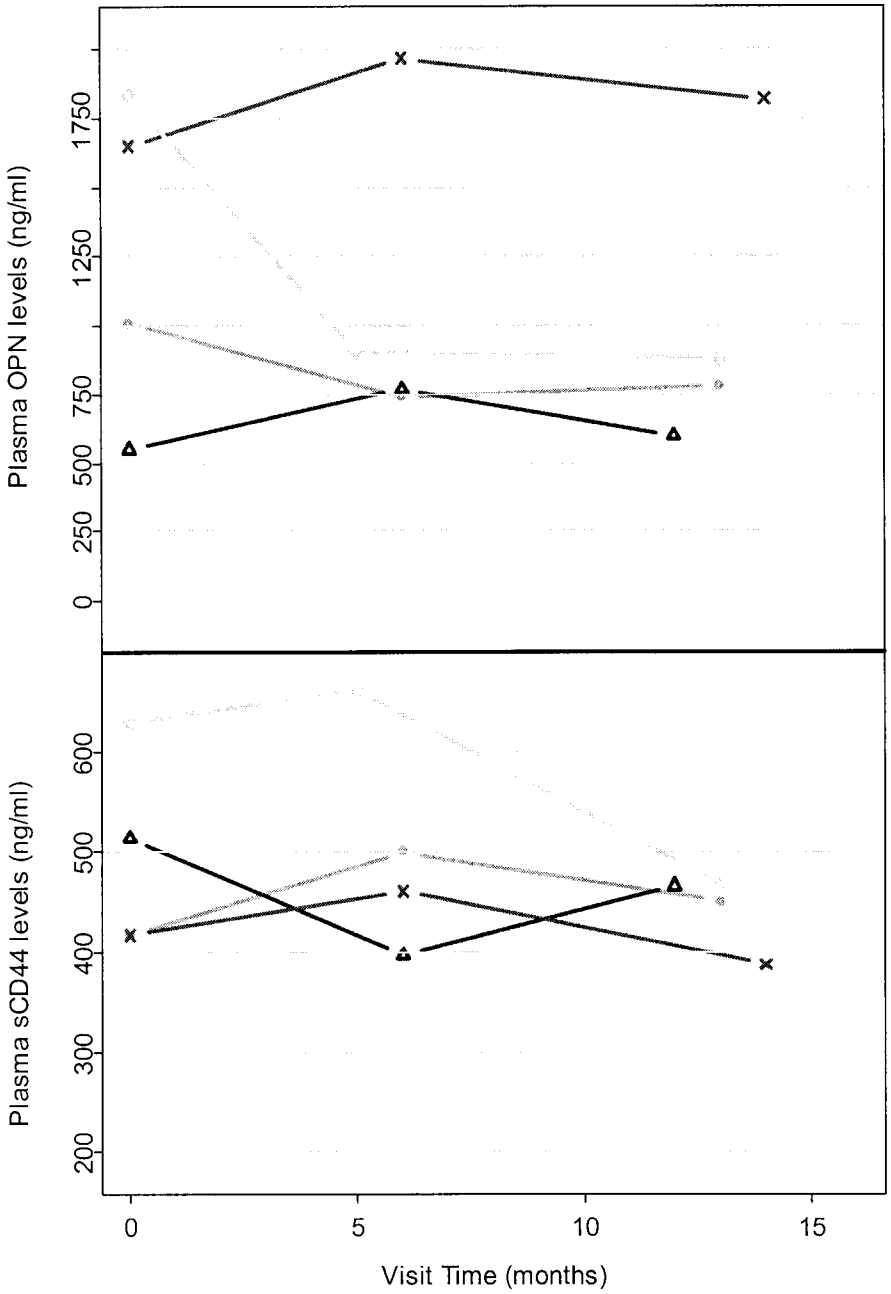
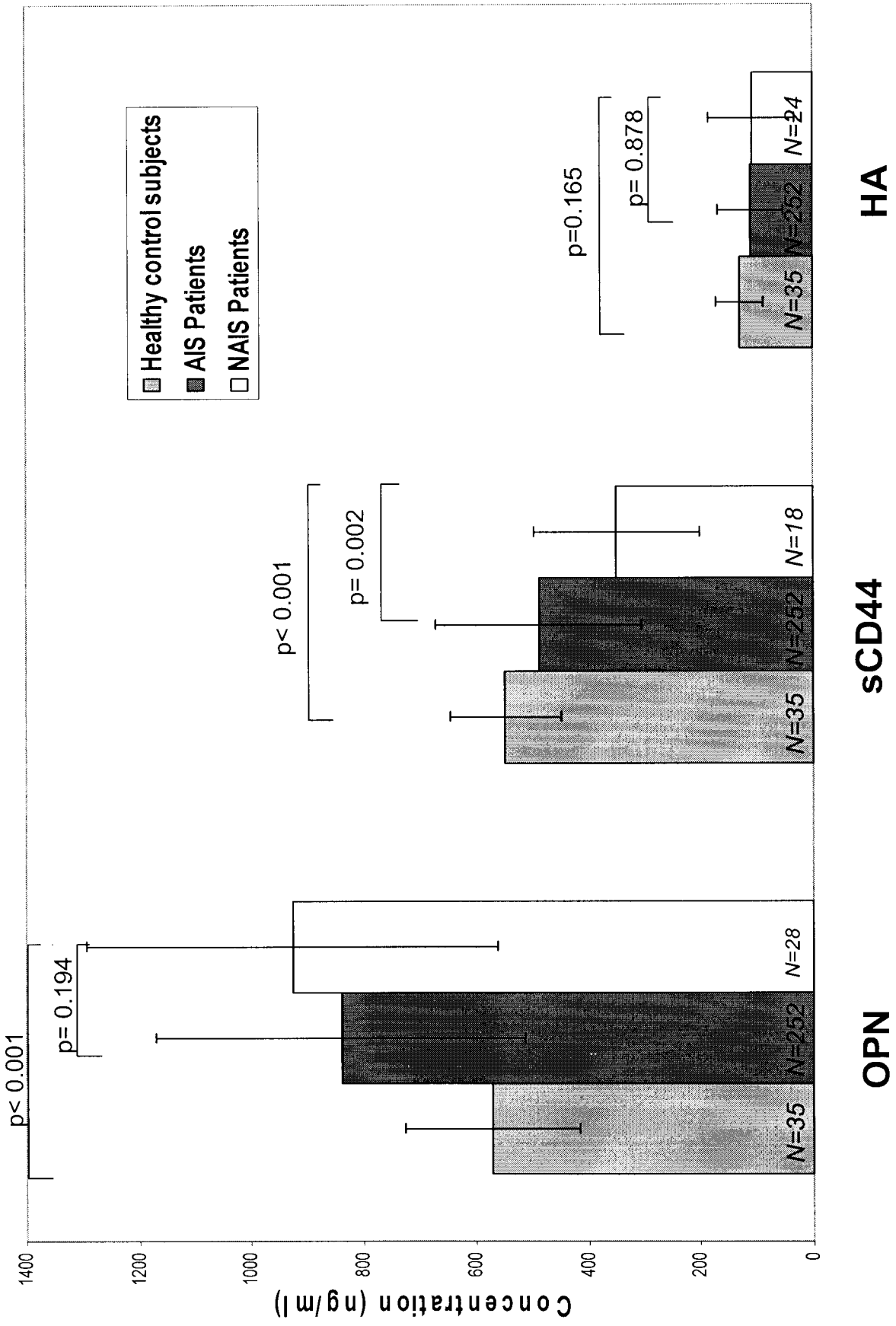

Plasma OPN levels (ng/ml)
0
250
500
750
1250
1750
Plasma sCD44 levels (ng/ml)
200
300
400
500
600
0
5
10
15
Visit Time (months)

Figure 11


Healthy control subjects
AIS Patients
NAIS Patients
Concentration (ng/ml)
1400
1200
1000
800
600
400
200
0
OPN
p< 0.001
p= 0.194
N=35
N=252
N=28
sCD44
p< 0.001
p= 0.002
N=35
N=252
N=18
HA
p=0.165
p= 0.878
N=35
N=252
N=24

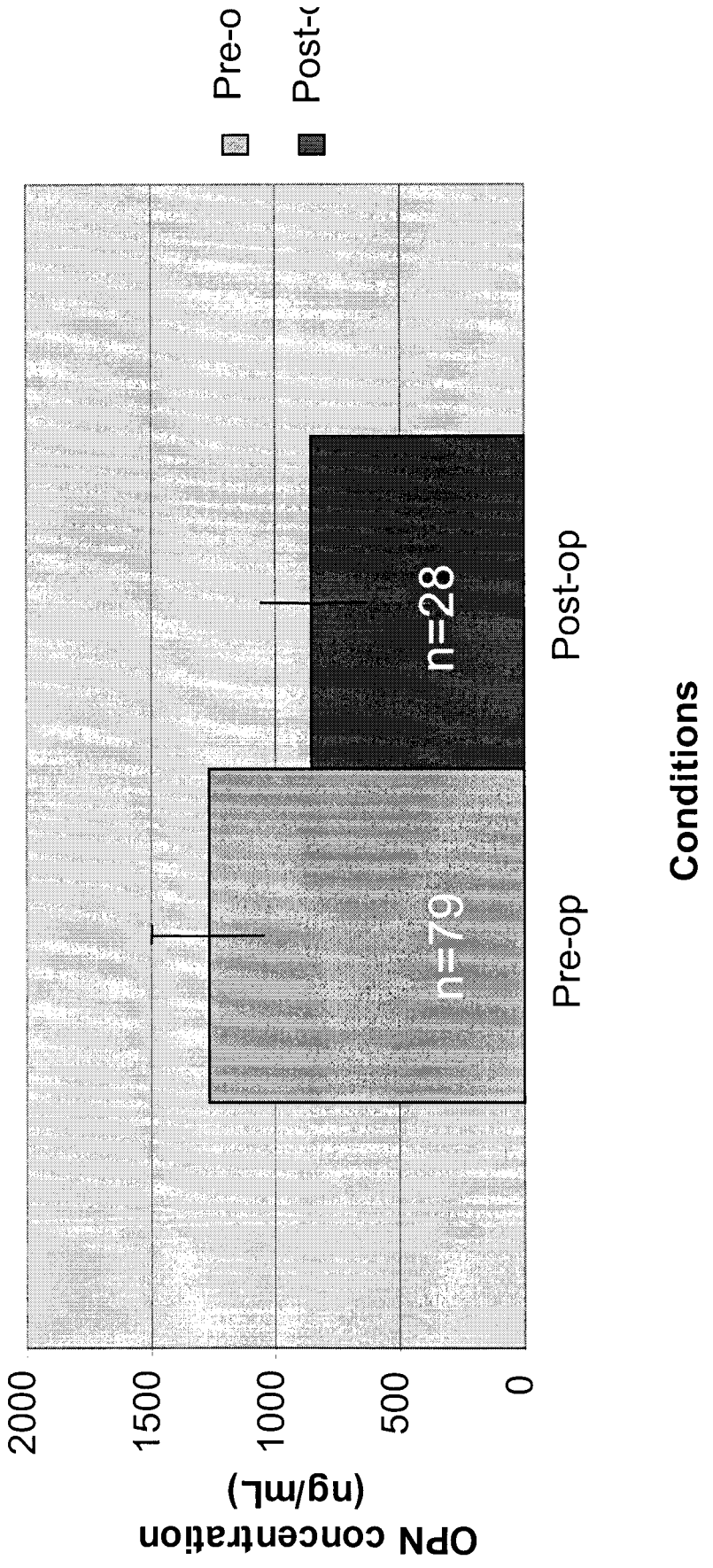

Pre-o
Post-
n=79
n=28
Pre-op
Post-op
Conditions
OPN concentration
(ng/mL)
2000
1500
1000
500
0

Figure 13

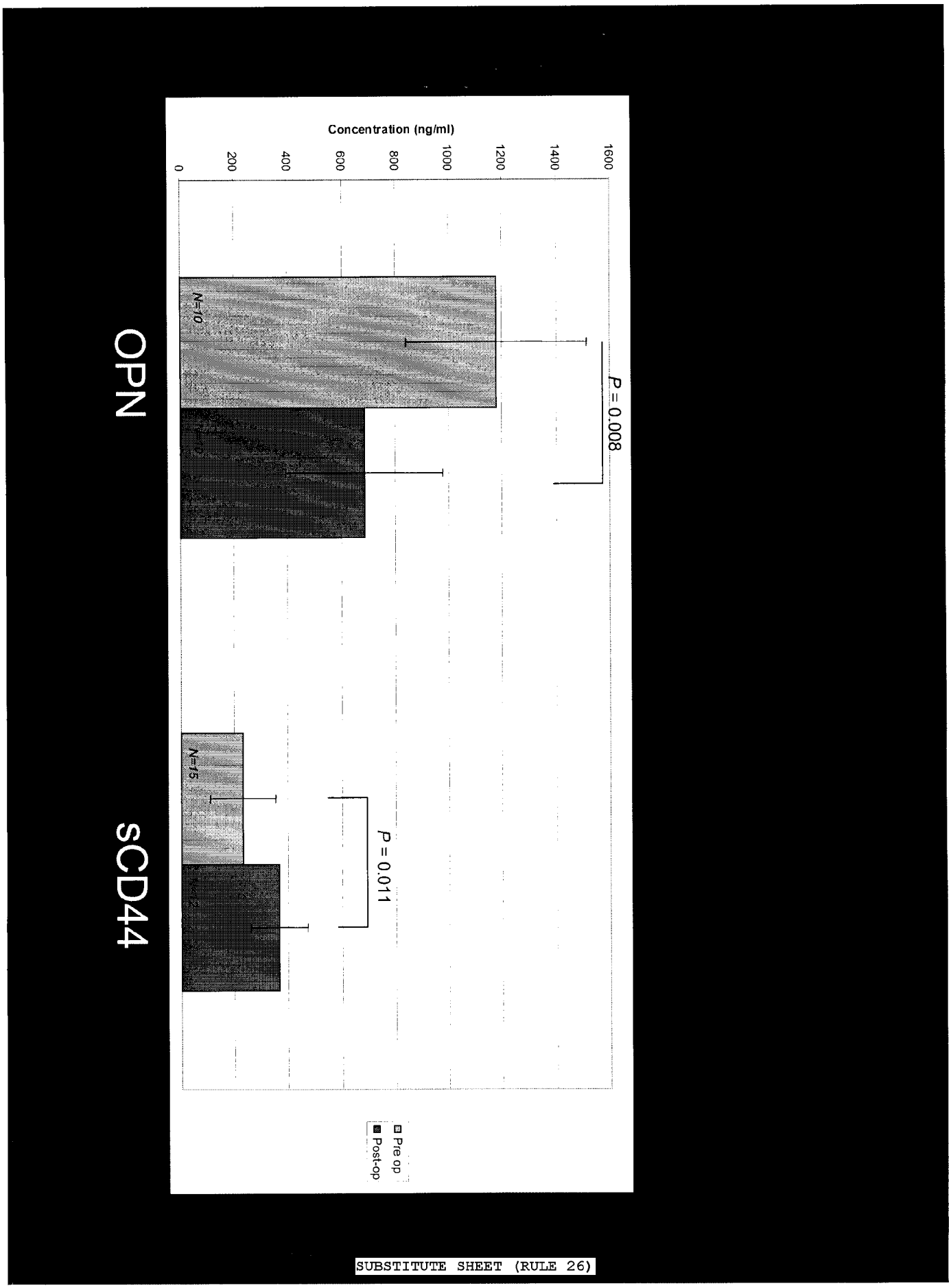
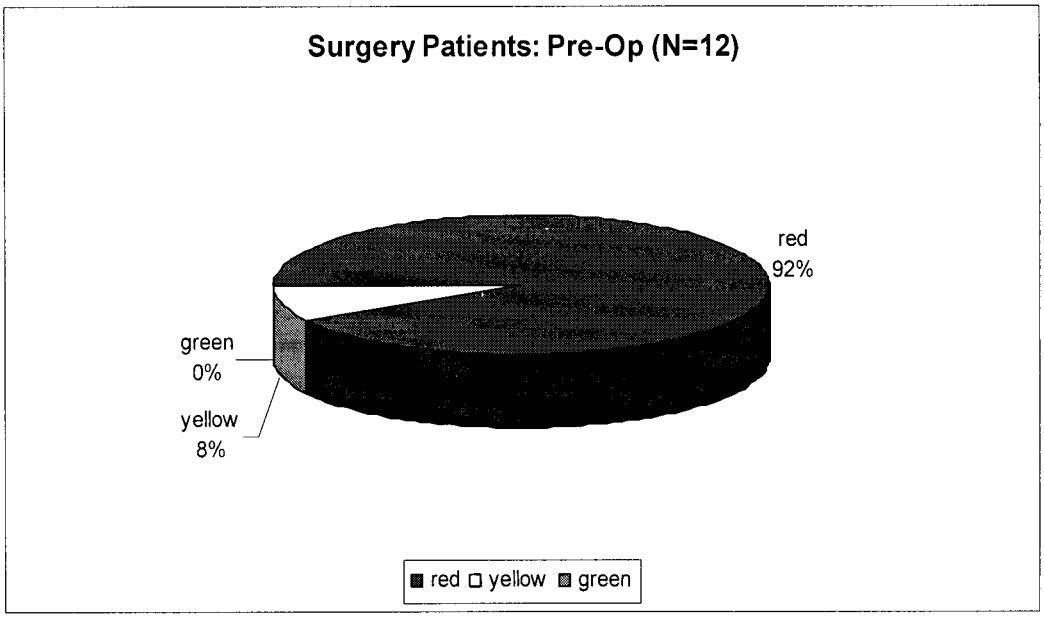


Figure 14

SUBSTITUTE SHEET (RULE 26)

A


Surgery Patients: Pre-Op (N=12)
red
92%
green
0%
yellow
8%
red yellow green

B

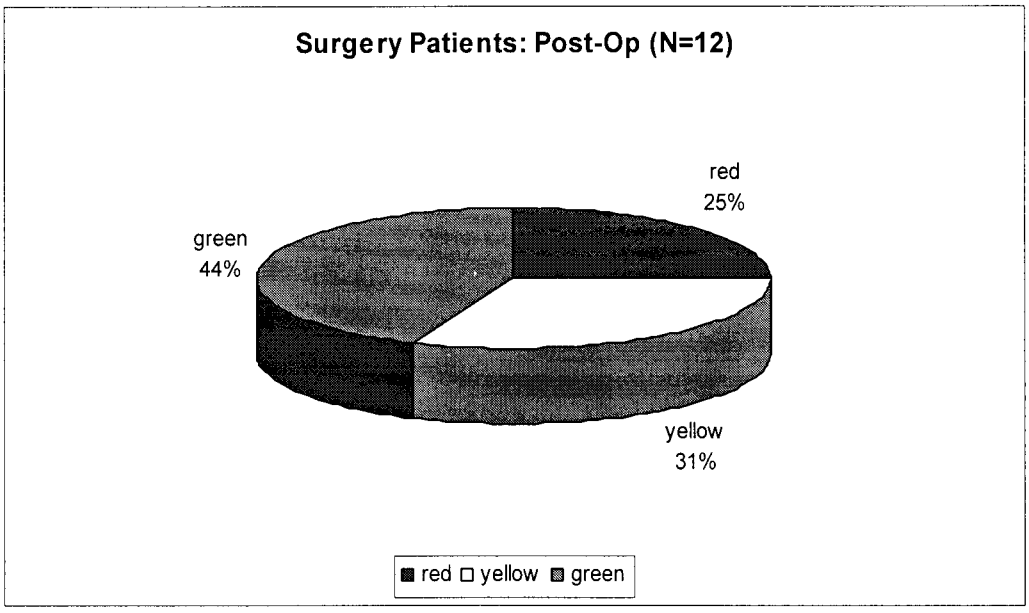
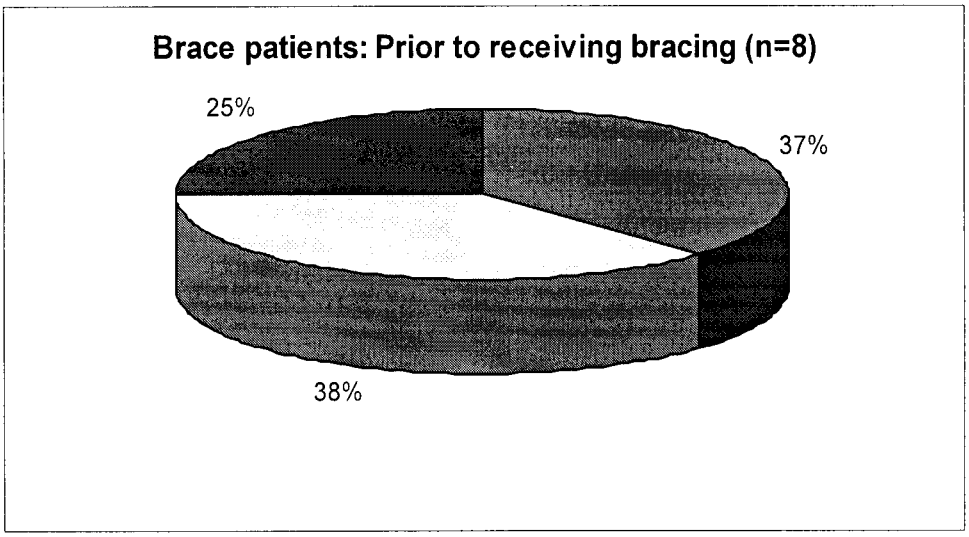

Surgery Patients: Post-Op (N=12)
red
25%
green
44%
yellow
31%
red yellow green

Figure 15

A


Brace patients: Prior to receiving bracing (n=8)
25%
37%
38%

B

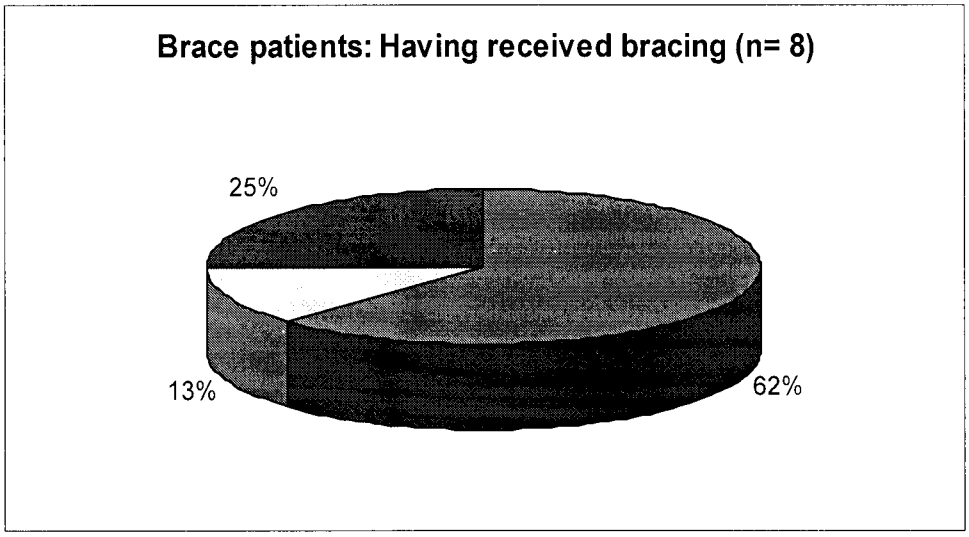

Brace patients: Having received bracing (n= 8)
25%
13%
62%

Figure 16

Figure 17

Figure 18

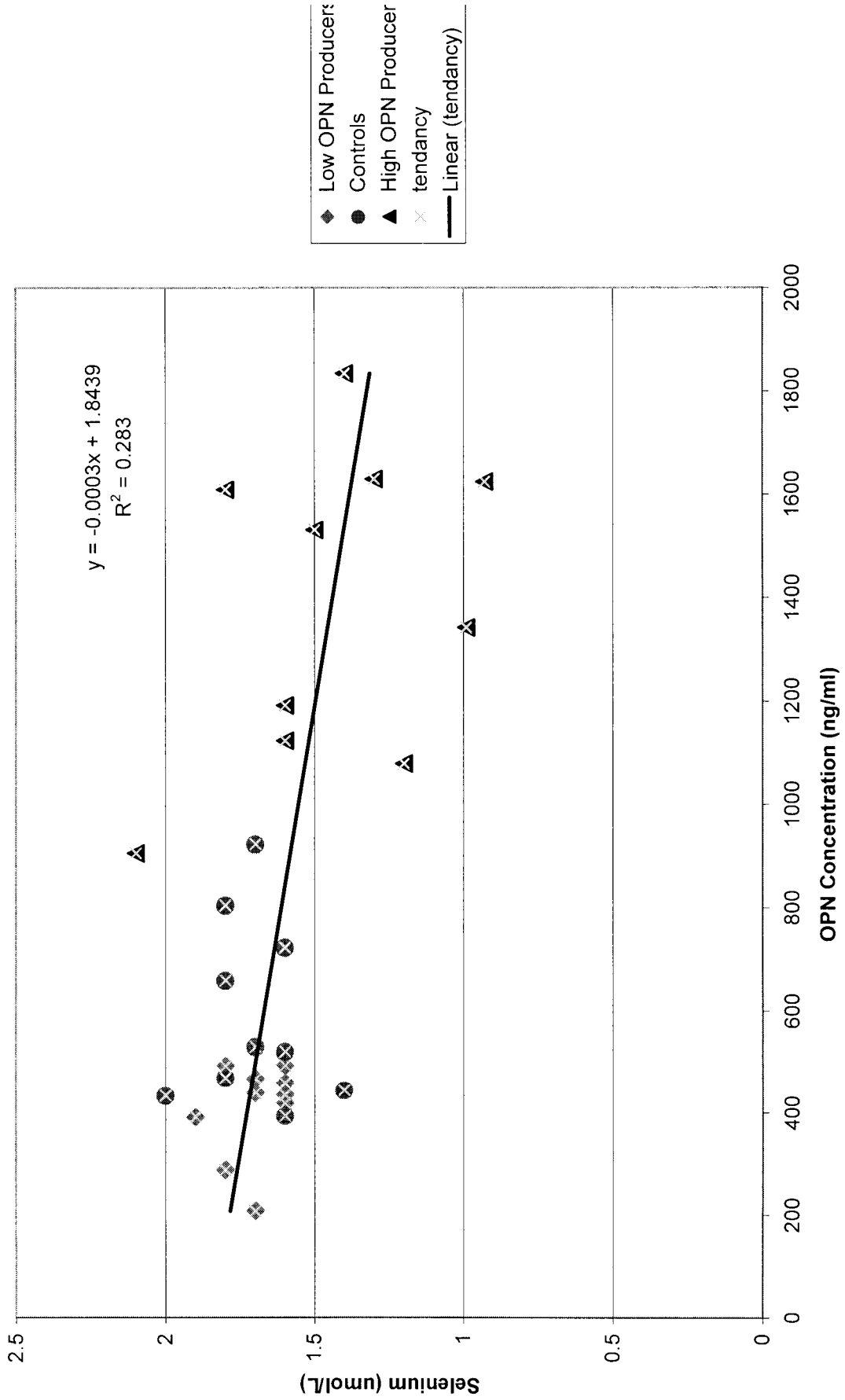
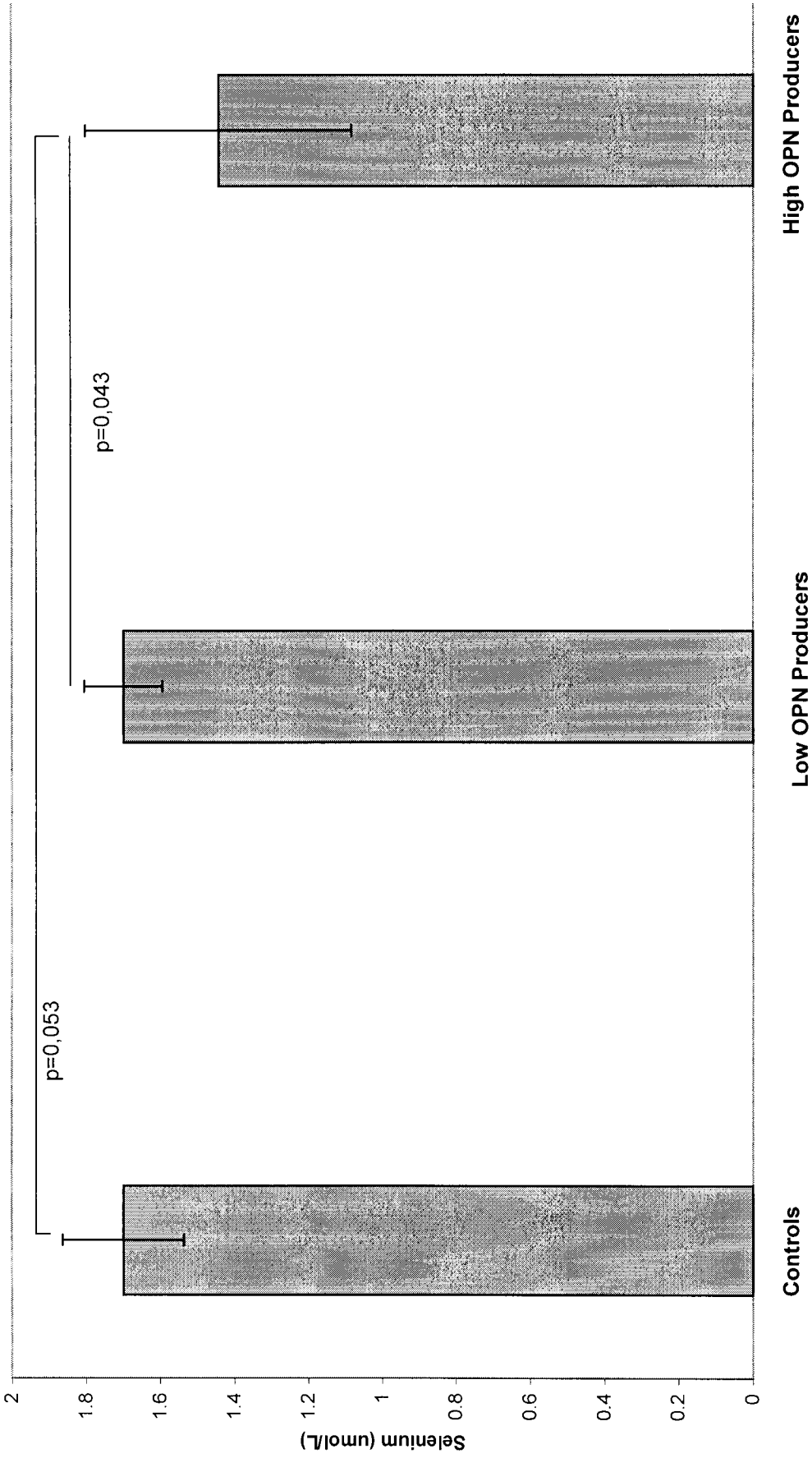

Low OPN Producers
Controls
High OPN Producer
tendancy
Linear (tendancy)
y = -0.0003x + 1.8439
R2 = 0.283
Selenium (umol/L)
OPN Concentration (ng/ml)
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
1200
1400
1600
1800
2000

Figure 19


p=0,053
p=0,043
Controls
Low OPN Producers
High OPN Producers
Selenium (umol/L)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

NM_001040058 transcript variant 1

```
   1 ctccctgtgt tggtggagga tgtctgcagc agcatttaaa ttctgggagg gcttggttgt
  61 cagcagcagc aggaggaggc agagcacagc atcgtcggga ccagactcgt ctcaggccag
 121 ttgcagcctt ctcagccaaa cgccgaccaa ggaaaactca ctaccatgag aattgcagtg
 181 atttgctttt gcctcctagg catcacctgt gccataccag ttaaacaggc tgattctgga
 241 agttctgagg aaaagcagct ttacaacaaa tacccagatg ctgtggccac atggctaaac
 301 cctgacccat ctcagaagca gaatctccta gccccacaga atgctgtgtc ctctgaagaa
 361 accaatgact ttaaacaaga gacccttcca agtaagtcca acgaaagcca tgaccacatg
 421 gatgatatgg atgatgaaga tgatgatgac catgtggaca gccaggactc cattgactcg
 481 aacgactctg atgatgtaga tgacactgat gattctcacc agtctgatga gtctcaccat
 541 tctgatgaat ctgatgaact ggtcactgat tttcccacgg acctgccagc aaccgaagtt
 601 ttcactccag ttgtccccac agtagacaca tatgatggcc gaggtgatag tgtggtttat
 661 ggactgaggt caaaatctaa gaagtttcgc agacctgaca tccagtaccc tgatgctaca
 721 gacgaggaca tcacctcaca catggaaagc gaggagttga atggtgcata caaggccatc
 781 cccgttgccc aggacctgaa cgcgccttct gattgggaca gccgtgggaa ggacagttat
 841 gaaacgagtc agctggatga ccagagtgct gaaacccaca gccacaagca gtccagatta
 901 tataagcgga aagccaatga tgagagcaat gagcattccg atgtgattga tagtcaggaa
 961 ctttccaaag tcagccgtga attccacagc catgaatttc acagccatga agatatgctg
1021 gttgtagacc ccaaaagtaa ggaagaagat aaacacctga aatttcgtat ttctcatgaa
1081 ttagatagtg catcttctga ggtcaattaa aaggagaaaa aatacaattt ctcactttgc
1141 atttagtcaa aagaaaaaat gctttatagc aaaatgaaag agaacatgaa atgcttcttt
1201 ctcagtttat tggttgaatg tgtatctatt tgagtctgga aataactaat gtgtttgata
1261 attagtttag tttgtggctt catggaaact ccctgtaaac taaaagcttc agggttatgt
1321 ctatgttcat tctatagaag aaatgcaaac tatcactgta ttttaatatt tgttattctc
1381 tcatgaatag aaatttatgt agaagcaaac aaaatacttt tacccactta aaaagagaat
1441 ataacatttt atgtcactat aatcttttgt tttttaagtt agtgtatatt ttgttgtgat
1501 tatctttttg tggtgtgaat aaatctttta tcttgaatgt aataagaatt tggtggtgtc
1561 aattgcttat ttgttttccc acggttgtcc agcaattaat aaaacataac ctttttttact
1621 gcctaaaaaa aaaaaaaaaa a
```

NM_000582 transcript variant 2

```
   1 ctccctgtgt tggtggagga tgtctgcagc agcatttaaa ttctgggagg gcttggttgt
  61 cagcagcagc aggaggaggc agagcacagc atcgtcggga ccagactcgt ctcaggccag
 121 ttgcagcctt ctcagccaaa cgccgaccaa ggaaaactca ctaccatgag aattgcagtg
 181 atttgctttt gcctcctagg catcacctgt gccataccag ttaaacaggc tgattctgga
 241 agttctgagg aaaagcagct ttacaacaaa tacccagatg ctgtggccac atggctaaac
 301 cctgacccat ctcagaagca gaatctccta gccccacaga cccttccaag taagtccaac
 361 gaaagccatg accacatgga tgatatggat gatgaagatg atgatgacca tgtggacagc
 421 caggactcca ttgactcgaa cgactctgat gatgtagatg acactgatga ttctcaccag
 481 tctgatgagt ctcaccattc tgatgaatct gatgaactgg tcactgattt tcccacggac
 541 ctgccagcaa ccgaagtttt cactccagtt gtccccacag tagacacata tgatggccga
 601 ggtgatagtg tggtttatgg actgaggtca aaatctaaga agtttcgcag acctgacatc
 661 cagtaccctg atgctacaga cgaggacatc acctcacaca tggaaagcga ggagttgaat
 721 ggtgcataca aggccatccc cgttgcccag gacctgaacg cgccttctga ttgggacagc
 781 cgtgggaagg acagttatga aacgagtcag ctggatgacc agagtgctga aacccacagc
 841 cacaagcagt ccagattata taagcggaaa gccaatgatg agagcaatga gcattccgat
 901 gtgattgata gtcaggaact ttccaaagtc agccgtgaat tccacagcca tgaatttcac
 961 agccatgaag atatgctggt tgtagacccc aaaagtaagg aagaagataa acacctgaaa
1021 tttcgtattt ctcatgaatt agatagtgca tcttctgagg tcaattaaaa ggagaaaaaa
1081 tacaatttct cactttgcat ttagtcaaaa gaaaaaatgc tttatagcaa aatgaaagag
1141 aacatgaaat gcttctttct cagtttattg gttgaatgtg tatctatttg agtctggaaa
1201 taactaatgt gtttgataat tagtttagtt tgtggcttca tggaaactcc ctgtaaacta
1261 aaagcttcag ggttatgtct atgttcattc tatagaagaa atgcaaacta tcactgtatt
1321 ttaatatttg ttattctctc atgaatagaa atttatgtag aagcaaacaa aatactttta
1381 cccacttaaa aagagaatat aacattttat gtcactataa tcttttgttt tttaagttag
1441 tgtatatttt gttgtgatta tctttttgtg gtgtgaataa atcttttatc ttgaatgtaa
1501 taagaatttg gtggtgtcaa ttgcttattt gttttcccac ggttgtccag caattaataa
1561 aacataacct tttttactgc ctaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa
```

Figure 20

NM_001040060 transcript variant 3

```
   1 ctccctgtgt tggtggagga tgtctgcagc agcatttaaa ttctgggagg gcttggttgt
  61 cagcagcagc aggaggaggc agagcacagc atcgtcggga ccagactcgt ctcaggccag
 121 ttgcagcctt ctcagccaaa cgccgaccaa ggaaaactca ctaccatgag aattgcagtg
 181 atttgctttt gcctcctagg catcacctgt gccataccag ttaaacaggc tgattctgga
 241 agttctgagg aaaagcagaa tgctgtgtcc tctgaagaaa ccaatgactt taaacaagag
 301 acccttccaa gtaagtccaa cgaaagccat gaccacatgg atgatatgga tgatgaagat
 361 gatgatgacc atgtggacag ccaggactcc attgactcga acgactctga tgatgtagat
 421 gacactgatg attctcacca gtctgatgag tctcaccatt ctgatgaatc tgatgaactg
 481 gtcactgatt ttcccacgga cctgccagca accgaagttt tcactccagt tgtccccaca
 541 gtagacacat atgatggccg aggtgatagt gtggtttatg gactgaggtc aaaatctaag
 601 aagtttcgca gacctgacat ccagtaccct gatgctacag acgaggacat cacctcacac
 661 atggaaagcg aggagttgaa tggtgcatac aaggccatcc ccgttgccca ggacctgaac
 721 gcgccttctg attgggacag ccgtgggaag gacagttatg aaacgagtca gctggatgac
 781 cagagtgctg aaacccacag ccacaagcag tccagattat ataagcggaa agccaatgat
 841 gagagcaatg agcattccga tgtgattgat agtcaggaac tttccaaagt cagccgtgaa
 901 ttccacagcc atgaatttca cagccatgaa gatatgctgg ttgtagaccc caaaagtaag
 961 gaagaagata aacacctgaa atttcgtatt tctcatgaat tagatagtgc atcttctgag
1021 gtcaattaaa aggagaaaaa atacaatttc tcactttgca tttagtcaaa agaaaaaatg
1081 ctttatagca aaatgaaaga gaacatgaaa tgcttctttc tcagtttatt ggttgaatgt
1141 gtatctattt gagtctggaa ataactaatg tgtttgataa ttagtttagt ttgtggcttc
1201 atggaaactc cctgtaaact aaaagcttca gggttatgtc tatgttcatt ctatagaaga
1261 aatgcaaact atcactgtat tttaatattt gttattctct catgaataga aatttatgta
1321 gaagcaaaca aaatactttt acccacttaa aaagagaata taacatttta tgtcactata
1381 atcttttgtt ttttaagtta gtgtatattt tgttgtgatt atctttttgt ggtgtgaata
1441 aatcttttat cttgaatgta ataagaattt ggtggtgtca attgcttatt tgttttccca
1501 cggttgtcca gcaattaata aaacataacc ttttttactg cctaaaaaaa aaaaaaaaaa
```

Figure 20 (Continued)

NP_001035147 isoform a

```
  1 mriavicfcl lgitcaipvk qadsgsseek qlynkypdav atwlnpdpsq kqnllapqna
 61 vsseetndfk qetlpsksne shdhmddmdd eddddhvdsq dsidsndsdd vddtddshqs
121 deshhsdesd elvtdfptdl patevftpvv ptvdtydgrg dsvvyglrsk skkfrrpdiq
181 ypdatdedit shmeseelng aykaipvaqd lnapsdwdsr gkdsyetsql ddqsaethsh
241 kqsrlykrka ndesnehsdv idsqelskvs refhshefhs hedmlvvdpk skeedkhlkf
301 risheldsas sevn
```

NP_000573 isoform b

```
  1 mriavicfcl lgitcaipvk qadsgsseek qlynkypdav atwlnpdpsq kqnllapqtl
 61 psksneshdh mddmddeddd dhvdsqdsid sndsddvddt ddshqsdesh hsdesdelvt
121 dfptdlpate vftpvvptvd tydgrgdsvv yglrskskkf rrpdiqypda tdeditshme
181 seelngayka ipvaqdlnap sdwdsrgkds yetsqlddqs aethshkqsr lykrkandes
241 nehsdvidsq elskvsrefh shefhshedm lvvdpkskee dkhlkfrish eldsassevn
```

NP_001035149 isoform c

```
  1 mriavicfcl lgitcaipvk qadsgsseek qnavsseetn dfkqetlpsk sneshdhmdd
 61 mddeddddhv dsqdsidsnd sddvddtdds hqsdeshhsd esdelvtdfp tdlpatevft
121 pvvptvdtyd grgdsvvygl rskskkfrrp diqypdatde ditshmesee lngaykaipv
181 aqdlnapsdw dsrgkdsyet sqlddqsaet hshkqsrlyk rkandesneh sdvidsqels
241 kvsrefhshe fhshedmlvv dpkskeedkh lkfrisheld sassevn
```

Figure 20 (Continued)

NM_000610 transcript variant 1

```
   1 gagaagaaag ccagtgcgtc tctgggcgca ggggccagtg gggctcggag gcacaggcac
  61 cccgcgacac tccaggttcc ccgacccacg tccctggcag ccccgattat ttacagcctc
 121 agcagagcac ggggcggggg cagaggggcc cgcccgggag ggctgctact tcttaaaacc
 181 tctgcgggct gcttagtcac agccccccct gcttgggtgt gtccttcgct cgctccctcc
 241 ctccgtctta ggtcactgtt ttcaacctcg aataaaaact gcagccaact tccgaggcag
 301 cctcattgcc cagcggaccc cagcctctgc caggttcggt ccgccatcct cgtcccgtcc
 361 tccgccggcc cctgccccgc gcccagggat cctccagctc ctttcgcccg cgccctccgt
 421 tcgctccgga caccatggac aagttttggt ggcacgcagc ctggggactc tgcctcgtgc
 481 cgctgagcct ggcgcagatc gatttgaata taacctgccg ctttgcaggt gtattccacg
 541 tggagaaaaa tggtcgctac agcatctctc ggacggaggc cgctgacctc tgcaaggctt
 601 tcaatagcac cttgcccaca atggcccaga tggagaaagc tctgagcatc ggatttgaga
 661 cctgcaggta tgggttcata gaagggcacg tggtgattcc ccggatccac cccaactcca
 721 tctgtgcagc aaacaacaca ggggtgtaca tcctcacatc caacacctcc cagtatgaca
 781 catattgctt caatgcttca gctccacctg aagaagattg tacatcagtc acagacctgc
 841 ccaatgcctt tgatggacca attaccataa ctattgttaa ccgtgatggc acccgctatg
 901 tccagaaagg agaatacaga acgaatcctg aagacatcta ccccagcaac cctactgatg
 961 atgacgtgag cagcggctcc tccagtgaaa ggagcagcac ttcaggaggt tacatctttt
1021 acacctttc tactgtacac cccatcccag acgaagacag tccctggatc accgacagca
1081 cagacagaat ccctgctacc actttgatga gcactagtgc tacagcaact gagacagcaa
1141 ccaagaggca agaaacctgg gattggtttt catggttgtt tctaccatca gagtcaaaga
1201 atcatcttca cacaacaaca caaatggctg gtacgtcttc aaataccatc tcagcaggct
1261 gggagccaaa tgaagaaaat gaagatgaaa gagacagaca cctcagtttt tctggatcag
1321 gcattgatga tgatgaagat tttatctcca gcaccatttc aaccacacca cgggcttttg
1381 accacacaaa acagaaccag gactggaccc agtggaaccc aagccattca aatccggaag
1441 tgctacttca gacaaccaca aggatgactg atgtagacag aaatggcacc actgcttatg
1501 aaggaaactg gaacccagaa gcacaccctc ccctcattca ccatgagcat catgaggaag
1561 aagagacccc acattctaca agcacaatcc aggcaactcc tagtagtaca acggaagaaa
1621 cagctaccca gaaggaacag tggtttggca acagatggca tgagggatat cgccaaacac
1681 ccaaagaaga ctcccattcg acaacaggga cagctgcagc ctcagctcat accagccatc
1741 caatgcaagg aaggacaaca ccaagcccag aggacagttc ctggactgat ttcttcaacc
1801 caatctcaca ccccatggga cgaggtcatc aagcaggaag aaggatggat atggactcca
1861 gtcatagtat aacgcttcag cctactgcaa atccaaacac aggtttggtg gaagatttgg
1921 acaggacagg acctctttca atgacaacgc agcagagtaa ttctcagagc ttctctacat
1981 cacatgaagg cttggaagaa gataaagacc atccaacaac ttctactctg acatcaagca
2041 ataggaatga tgtcacaggt ggaagaagag acccaaatca ttctgaaggc tcaactactt
2101 tactggaagg ttatacctct cattacccac acacgaagga aagcaggacc ttcatcccag
2161 tgacctcagc taagactggg tcctttggag ttactgcagt tactgttgga gattccaact
2221 ctaatgtcaa tcgttcctta tcaggagacc aagacacatt ccaccccagt ggggggtccc
2281 ataccactca tggatctgaa tcagatggac actcacatgg gagtcaagaa ggtggagcaa
2341 acacaacctc tggtcctata aggacacccc aaattccaga atggctgatc atcttggcat
2401 ccctcttggc cttggctttg attcttgcag tttgcattgc agtcaacagt cgaagaaggt
2461 gtgggcagaa gaaaaagcta gtgatcaaca gtggcaatgg agctgtggag gacagaaagc
2521 caagtggact caacggagag gccagcaagt ctcaggaaat ggtgcatttg gtgaacaagg
2581 agtcgtcaga aactccagac cagtttatga cagctgatga gacaaggaac ctgcagaatg
2641 tggacatgaa gattggggtg taacacctac accattatct tggaaagaaa caaccgttgg
2701 aaacataacc attacaggga gctgggacac ttaacagatg caatgtgcta ctgattgttt
2761 cattgcgaat ctttttttagc ataaaatttt ctactctttt tgtttttttgt gttttgttct
2821 ttaaagtcag gtccaatttg taaaaacagc attgctttct gaaattaggg cccaattaat
2881 aatcagcaag aatttgatcg ttccagttcc cacttggagg cctttcatcc ctcgggtgtg
2941 ctatggatgg cttctaacaa aaactacaca tatgtattcc tgatcgccaa cctttccccc
3001 accagctaag gacatttccc agggttaata gggcctggtc cctgggagga aatttgaatg
3061 ggtccatttt gcccttccat agcctaatcc ctgggcattg ctttccactg aggttggggg
3121 ttggggtgta ctagttacac atcttcaaca gacccctct agaaattttt cagatgcttc
3181 tgggagacac ccaaagggtg aagctattta tctgtagtaa actatttatc tgtgtttttg
3241 aaatattaaa ccctggatca gtcctttgat cagtataatt ttttaaagtt actttgtcag
3301 aggcacaaaa gggtttaaac tgattcataa taaatatctg tacttcttcg atcttcacct
3361 tttgtgctgt gattcttcag tttctaaacc agcactgtct gggtccctac aatgtatcag
```

Figure 21

```
3421 gaagagctga gaatggtaag gagactcttc taagtcttca tctcagagac cctgagttcc
3481 cactcagacc cactcagcca aatctcatgg aagaccaagg agggcagcac tgtttttgtt
3541 ttttgttttt tgtttttttt ttttgacact gtccaaaggt tttccatcct gtcctggaat
3601 cagagttgga agctgaggag cttcagcctc ttttatggtt taatggccac ctgttctctc
3661 ctgtgaaagg ctttgcaaag tcacattaag tttgcatgac ctgttatccc tggggcccta
3721 tttcatagag gctggcccta ttagtgattt ccaaaaacaa tatggaagtg ccttttgatg
3781 tcttacaata agagaagaag ccaatggaaa tgaaagagat tggcaaaggg gaaggatgat
3841 gccatgtaga tcctgtttga catttttatg gctgtatttg taaacttaaa cacaccagtg
3901 tctgttcttg atgcagttgc tatttaggat gagttaagtg cctggggagt ccctcaaaag
3961 gttaaaggga ttcccatcat tggaatctta tcaccagata ggcaagttta tgaccaaaca
4021 agagagtact ggctttatcc tctaacctca tattttctcc cacttggcaa gtcctttgtg
4081 gcatttattc atcagtcagg gtgtccgatt ggtcctagaa cttccaaagg ctgcttgtca
4141 tagaagccat tgcatctata aagcaacggc tcctgttaaa tggtatctcc tttctgaggc
4201 tcctactaaa agtcatttgt tacctaaact tatgtgctta acaggcaatg cttctcagac
4261 cacaaagcag aaagaagaag aaaagctcct gactaaatca gggctgggct tagacagagt
4321 tgatctgtag aatatcttta aaggagagat gtcaactttc tgcactattc ccagcctctg
4381 ctcctccctg tctaccctct cccctccctc tctccctcca cttcacccca caatcttgaa
4441 aaacttcctt tctcttctgt gaacatcatt ggccagatcc attttcagtg gtctggattt
4501 ctttttattt tcttttcaac ttgaaagaaa ctggacatta ggccactatg tgttgttact
4561 gccactagtg ttcaagtgcc tcttgttttc ccagagattt cctgggtctg ccagaggccc
4621 agacaggctc actcaagctc tttaactgaa aagcaacaag ccactccagg acaaggttca
4681 aaatggttac aacagcctct acctgtcgcc ccagggagaa aggggtagtg atacaagtct
4741 catagccaga gatggttttc cactccttct agatattccc aaaaagaggc tgagacagga
4801 ggttattttc aattttattt tggaattaaa tacttttttc cctttattac tgttgtagtc
4861 cctcacttgg atatacctct gttttcacga tagaaataag ggaggtctag agcttctatt
4921 ccttggccat tgtcaacgga gagctggcca agtcttcaca aacccttgca acattgcctg
4981 aagtttatgg aataagatgt attctcactc ccttgatctc aagggcgtaa ctctggaagc
5041 acagcttgac tacacgtcat ttttaccaat gattttcagg tgacctgggc taagtcattt
5101 aaactgggtc tttataaaag taaaaggcca acatttaatt attttgcaaa gcaacctaag
5161 agctaaagat gtaatttttc ttgcaattgt aaatcttttg tgtctcctga agacttccct
5221 taaaattagc tctgagtgaa aaatcaaaag agacaaaaga catcttcgaa tccatatttc
5281 aagcctggta gaattggctt ttctagcaga acctttccaa aagttttata ttgagattca
5341 taacaacacc aagaattgat tttgtagcca acattcattc aatactgtta tatcagagga
5401 gtaggagaga ggaaacattt gacttatctg gaaaagcaaa atgtacttaa gaataagaat
5461 aacatggtcc attcaccttt atgttataga tatgtctttg tgtaaatcat ttgttttgag
5521 ttttcaaaga atagcccatt gttcattctt gtgctgtaca atgaccactg ttattgttac
5581 tttgactttt cagagcacac ccttcctctg gtttttgtat atttattgat ggatcaataa
5641 taatgaggaa agcatgatat gtatattgct gagttgaaag cacttattgg aaaatattaa
5701 aaggctaaca ttaaaagact aaaggaaaca gaaaaaaaaa aaaaaaaa
```

Figure 21 (continued)

NM_001001389 transcript variant 2

```
   1 gagaagaaag ccagtgcgtc tctgggcgca ggggccagtg gggctcggag gcacaggcac
  61 cccgcgacac tccaggttcc ccgacccacg tccctggcag ccccgattat ttacagcctc
 121 agcagagcac ggggcggggg cagaggggcc cgcccgggag ggctgctact tcttaaaacc
 181 tctgcgggct gcttagtcac agccccccct gcttgggtgt gtccttcgct cgctccctcc
 241 ctccgtctta ggtcactgtt ttcaacctcg aataaaaact gcagccaact tccgaggcag
 301 cctcattgcc cagcggaccc cagcctctgc caggttcggt ccgccatcct cgtcccgtcc
 361 tccgccggcc cctgccccgc gcccagggat cctccagctc ctttcgcccg cgccctccgt
 421 tcgctccgga caccatggac aagttttggt ggcacgcagc ctggggactc tgcctcgtgc
 481 cgctgagcct ggcgcagatc gatttgaata taacctgccg ctttgcaggt gtattccacg
 541 tggagaaaaa tggtcgctac agcatctctc ggacggaggc cgctgacctc tgcaaggctt
 601 tcaatagcac cttgcccaca atggcccaga tggagaaagc tctgagcatc ggatttgaga
 661 cctgcaggta tgggttcata gaagggcacg tggtgattcc ccggatccac cccaactcca
 721 tctgtgcagc aaacaacaca ggggtgtaca tcctcacatc caacacctcc cagtatgaca
 781 catattgctt caatgcttca gctccacctg aagaagattg tacatcagtc acagacctgc
 841 ccaatgcctt tgatggacca attaccataa ctattgttaa ccgtgatggc acccgctatg
 901 tccagaaagg agaatacaga acgaatcctg aagacatcta ccccagcaac cctactgatg
 961 atgacgtgag cagcggctcc tccagtgaaa ggagcagcac ttcaggaggt tacatctttt
1021 acacctttc tactgtacac cccatcccag acgaagacag tccctggatc accgacagca
1081 cagacagaat ccctgctacc agtacgtctt caaataccat ctcagcaggc tgggagccaa
1141 atgaagaaaa tgaagatgaa agagacagac acctcagttt ttctggatca ggcattgatg
1201 atgatgaaga ttttatctcc agcaccattt caaccacacc acgggctttt gaccacacaa
1261 aacagaacca ggactggacc cagtggaacc caagccattc aaatccggaa gtgctacttc
1321 agacaaccac aaggatgact gatgtagaca gaaatggcac cactgcttat gaaggaaact
1381 ggaacccaga agcacaccct cccctcattc accatgagca tcatgaggaa gaagagaccc
1441 cacattctac aagcacaatc caggcaactc ctagtagtac aacggaagaa acagctaccc
1501 agaaggaaca gtggtttggc aacagatggc atgagggata tcgccaaaca cccaaagaag
1561 actcccattc gacaacaggg acagctgcag cctcagctca taccagccat ccaatgcaag
1621 gaaggacaac accaagccca gaggacagtt cctggactga tttcttcaac ccaatctcac
1681 accccatggg acgaggtcat caagcaggaa gaaggatgga tatggactcc agtcatagta
1741 taacgcttca gcctactgca aatccaaaca caggtttggt ggaagatttg gacaggacag
1801 gacctctttc aatgacaacg cagcagagta attctcagag cttctctaca tcacatgaag
1861 gcttggaaga agataaagac catccaacaa cttctactct gacatcaagc aataggaatg
1921 atgtcacagg tggaagaaga gacccaaatc attctgaagg ctcaactact ttactggaag
1981 gttatacctc tcattaccca cacacgaagg aaagcaggac cttcatccca gtgacctcag
2041 ctaagactgg gtcctttgga gttactgcag ttactgttgg agattccaac tctaatgtca
2101 atcgttcctt atcaggagac caagacacat tccaccccag tgggggggtcc cataccactc
2161 atggatctga atcagatgga cactcacatg ggagtcaaga aggtggagca aacacaacct
2221 ctggtcctat aaggacaccc caaattccag aatggctgat catcttggca tccctcttgg
2281 ccttggcttt gattcttgca gtttgcattg cagtcaacag tcgaagaagg tgtgggcaga
2341 agaaaaagct agtgatcaac agtggcaatg gagctgtgga ggacagaaag ccaagtggac
2401 tcaacggaga ggccagcaag tctcaggaaa tggtgcattt ggtgaacaag gagtcgtcag
2461 aaactccaga ccagtttatg acagctgatg agacaaggaa cctgcagaat gtggacatga
2521 agattggggt gtaacaccta caccattatc ttggaaagaa acaaccgttg gaaacataac
2581 cattacaggg agctgggaca cttaacagat gcaatgtgct actgattgtt tcattgcgaa
2641 tctttttag cataaaattt tctactcttt ttgttttttg tgttttgttc tttaaagtca
2701 ggtccaattt gtaaaaacag cattgctttc tgaaattagg gcccaattaa taatcagcaa
2761 gaatttgatc gttccagttc ccacttggag gcctttcatc cctcgggtgt gctatggatg
2821 gcttctaaca aaaactacac atatgtattc ctgatcgcca acctttcccc caccagctaa
2881 ggacatttcc cagggttaat agggcctggt ccctgggagg aaatttgaat gggtccattt
2941 tgcccttcca tagcctaatc cctgggcatt gctttccact gaggttgggg gttggggtgt
3001 actagttaca catcttcaac agacccccctc tagaaatttt tcagatgctt ctgggagaca
3061 cccaaagggt gaagctattt atctgtagta aactatttat ctgtgttttt gaaatattaa
3121 accctggatc agtcctttga tcagtataat tttttaaagt tactttgtca gaggcacaaa
3181 agggtttaaa ctgattcata ataaatatct gtacttcttc gatcttcacc ttttgtgctg
3241 tgattcttca gtttctaaac cagcactgtc tgggtcccta caatgtatca ggaagagctg
3301 agaatggtaa ggagactctt ctaagtcttc atctcagaga ccctgagttc ccactcagac
3361 ccactcagcc aaatctcatg gaagaccaag gagggcagca ctgtttttgt tttttgtttt
```

Figure 21 (continued)

```
3421 ttgttttttt tttttgacac tgtccaaagg ttttccatcc tgtcctggaa tcagagttgg
3481 aagctgagga gcttcagcct cttttatggt ttaatggcca cctgttctct cctgtgaaag
3541 gctttgcaaa gtcacattaa gtttgcatga cctgttatcc ctggggccct atttcataga
3601 ggctggccct attagtgatt tccaaaaaca atatggaagt gccttttgat gtcttacaat
3661 aagagaagaa gccaatggaa atgaaagaga ttggcaaagg ggaaggatga tgccatgtag
3721 atcctgtttg acatttttat ggctgtattt gtaaacttaa acacaccagt gtctgttctt
3781 gatgcagttg ctatttagga tgagttaagt gcctggggag tccctcaaaa ggttaaaggg
3841 attcccatca ttggaatctt atcaccagat aggcaagttt atgaccaaac aagagagtac
3901 tggctttatc ctctaacctc atattttctc ccacttggca agtcctttgt ggcatttatt
3961 catcagtcag ggtgtccgat tggtcctaga acttccaaag gctgcttgtc atagaagcca
4021 ttgcatctat aaagcaacgg ctcctgttaa atggtatctc ctttctgagg ctcctactaa
4081 aagtcatttg ttacctaaac ttatgtgctt aacaggcaat gcttctcaga ccacaaagca
4141 gaaagaagaa gaaaagctcc tgactaaatc agggctgggc ttagacagag ttgatctgta
4201 gaatatcttt aaaggagaga tgtcaacttt ctgcactatt cccagcctct gctcctccct
4261 gtctaccctc tcccctccct ctctccctcc acttcacccc acaatcttga aaaacttcct
4321 ttctcttctg tgaacatcat tggccagatc cattttcagt ggtctggatt tctttttatt
4381 ttcttttcaa cttgaaagaa actggacatt aggccactat gtgttgttac tgccactagt
4441 gttcaagtgc ctcttgtttt cccagagatt tcctgggtct gccagaggcc cagacaggct
4501 cactcaagct ctttaactga aaagcaacaa gccactccag gacaaggttc aaaatggtta
4561 caacagcctc tacctgtcgc cccagggaga aaggggtagt gatacaagtc tcatagccag
4621 agatggtttt ccactccttc tagatattcc caaaaagagg ctgagacagg aggttatttt
4681 caattttatt ttggaattaa atacttttt ccctttatta ctgttgtagt ccctcacttg
4741 gatatacctc tgttttcacg atagaaataa gggaggtcta gagcttctat tccttggcca
4801 ttgtcaacgg agagctggcc aagtcttcac aaacccttgc aacattgcct gaagtttatg
4861 gaataagatg tattctcact cccttgatct caagggcgta actctggaag cacagcttga
4921 ctacacgtca tttttaccaa tgattttcag gtgacctggg ctaagtcatt taaactgggt
4981 ctttataaaa gtaaaaggcc aacatttaat tattttgcaa agcaacctaa gagctaaaga
5041 tgtaattttt cttgcaattg taaatctttt gtgtctcctg aagacttccc ttaaaattag
5101 ctctgagtga aaaatcaaaa gagacaaaag acatcttcga atccatattt caagcctggt
5161 agaattggct tttctagcag aacctttcca aaagttttat attgagattc ataacaacac
5221 caagaattga ttttgtagcc aacattcatt caatactgtt atatcagagg agtaggagag
5281 aggaaacatt tgacttatct ggaaaagcaa aatgtactta agaataagaa taacatggtc
5341 cattcacctt tatgttatag atatgtcttt gtgtaaatca tttgttttga gttttcaaag
5401 aatagcccat tgttcattct tgtgctgtac aatgaccact gttattgtta ctttgacttt
5461 tcagagcaca cccttcctct ggtttttgta tatttattga tggatcaata ataatgagga
5521 aagcatgata tgtatattgc tgagttgaaa gcacttattg gaaaatatta aaaggctaac
5581 attaaaagac taaaggaaac agaaaaaaaa aaaaaaaaa
```

Figure 21 (continued)

NM_001001390 transcript variant 3

```
   1 gagaagaaag ccagtgcgtc tctgggcgca ggggccagtg gggctcggag gcacaggcac
  61 cccgcgacac tccaggttcc ccgacccacg tccctggcag ccccgattat ttacagcctc
 121 agcagagcac ggggcggggg cagaggggcc cgcccgggag ggctgctact tcttaaaacc
 181 tctgcgggct gcttagtcac agccccccct gcttgggtgt gtccttcgct cgctccctcc
 241 ctccgtctta ggtcactgtt ttcaacctcg aataaaaact gcagccaact tccgaggcag
 301 cctcattgcc cagcggaccc cagcctctgc caggttcggt ccgccatcct cgtcccgtcc
 361 tccgccggcc cctgccccgc gcccagggat cctccagctc ctttcgcccg cgccctccgt
 421 tcgctccgga caccatggac aagttttggt ggcacgcagc ctggggactc tgcctcgtgc
 481 cgctgagcct ggcgcagatc gatttgaata taacctgccg ctttgcaggt gtattccacg
 541 tggagaaaaa tggtcgctac agcatctctc ggacggaggc cgctgacctc tgcaaggctt
 601 tcaatagcac cttgcccaca atggcccaga tggagaaagc tctgagcatc ggatttgaga
 661 cctgcaggta tgggttcata gaagggcacg tggtgattcc ccggatccac cccaactcca
 721 tctgtgcagc aaacaacaca ggggtgtaca tcctcacatc caacacctcc cagtatgaca
 781 catattgctt caatgcttca gctccacctg aagaagattg tacatcagtc acagacctgc
 841 ccaatgcctt tgatggacca attaccataa ctattgttaa ccgtgatggc acccgctatg
 901 tccagaaagg agaatacaga acgaatcctg aagacatcta ccccagcaac cctactgatg
 961 atgacgtgag cagcggctcc tccagtgaaa ggagcagcac ttcaggaggt tacatctttt
1021 acacctttc tactgtacac cccatcccag acgaagacag tccctggatc accgacagca
1081 cagacagaat ccctgctacc aatatggact ccagtcatag tataacgctt cagcctactg
1141 caaatccaaa cacaggtttg gtggaagatt tggacaggac aggacctctt tcaatgacaa
1201 cgcagcagag taattctcag agcttctcta catcacatga aggcttggaa gaagataaag
1261 accatccaac aacttctact ctgacatcaa gcaataggaa tgatgtcaca ggtggaagaa
1321 gagacccaaa tcattctgaa ggctcaacta ctttactgga aggttatacc tctcattacc
1381 cacacacgaa ggaaagcagg accttcatcc cagtgacctc agctaagact gggtcctttg
1441 gagttactgc agttactgtt ggagattcca actctaatgt caatcgttcc ttatcaggag
1501 accaagacac attccacccc agtgggggt cccataccac tcatggatct gaatcagatg
1561 gacactcaca tgggagtcaa gaaggtggag caaacacaac ctctggtcct ataaggacac
1621 cccaaattcc agaatggctg atcatcttgg catccctctt ggccttggct ttgattcttg
1681 cagtttgcat tgcagtcaac agtcgaagaa ggtgtgggca gaagaaaaag ctagtgatca
1741 acagtggcaa tggagctgtg gaggacagaa agccaagtgg actcaacgga gaggccagca
1801 agtctcagga aatggtgcat ttggtgaaca aggagtcgtc agaaactcca gaccagttta
1861 tgacagctga tgagacaagg aacctgcaga atgtggacat gaagattggg gtgtaacacc
1921 tacaccatta tcttggaaag aaacaaccgt tggaaacata accattacag ggagctggga
1981 cacttaacag atgcaatgtg ctactgattg tttcattgcg aatctttttt agcataaaat
2041 tttctactct ttttgttttt tgtgttttgt tctttaaagt caggtccaat ttgtaaaaac
2101 agcattgctt tctgaaatta gggcccaatt aataatcagc aagaatttga tcgttccagt
2161 tcccacttgg aggcctttca tccctcgggt gtgctatgga tggcttctaa caaaaactac
2221 acatatgtat tcctgatcgc caacctttcc cccaccagct aaggacattt cccagggtta
2281 atagggcctg gtccctggga ggaaatttga atgggtccat tttgcccttc catagcctaa
2341 tccctgggca ttgctttcca ctgaggttgg gggttggggt gtactagtta cacatcttca
2401 acagaccccc tctagaaatt tttcagatgc ttctgggaga cacccaaagg gtgaagctat
2461 ttatctgtag taaactattt atctgtgttt ttgaaatatt aaaccctgga tcagtccttt
2521 gatcagtata attttttaaa gttactttgt cagaggcaca aaagggttta aactgattca
2581 taataaatat ctgtacttct tcgatcttca ccttttgtgc tgtgattctt cagtttctaa
2641 accagcactg tctgggtccc tacaatgtat caggaagagc tgagaatggt aaggagactc
2701 ttctaagtct tcatctcaga gaccctgagt tcccactcag acccactcag ccaaatctca
2761 tggaagacca aggagggcag cactgttttt gttttttgtt ttttgttttt ttttttgac
2821 actgtccaaa ggttttccat cctgtcctgg aatcagagtt ggaagctgag gagcttcagc
2881 ctcttttatg gtttaatggc cacctgttct ctcctgtgaa aggctttgca aagtcacatt
2941 aagtttgcat gacctgttat ccctggggcc ctatttcata gaggctggcc ctattagtga
3001 tttccaaaaa caatatggaa gtgccttttg atgtcttaca ataagagaag aagccaatgg
3061 aaatgaaaga gattggcaaa ggggaaggat gatgccatgt agatcctgtt tgacattttt
3121 atggctgtat ttgtaaactt aaacacacca gtgtctgttc ttgatgcagt tgctatttag
3181 gatgagttaa gtgcctgggg agtccctcaa aaggttaaag ggattcccat cattggaatc
3241 ttatcaccag ataggcaagt ttatgaccaa acaagagagt actggcttta tcctctaacc
3301 tcatattttc tcccacttgg caagtccttt gtggcattta ttcatcagtc agggtgtccg
```

Figure 21 (continued)

```
3361 attggtccta gaacttccaa aggctgcttg tcatagaagc cattgcatct ataaagcaac
3421 ggctcctgtt aaatggtatc tcctttctga ggctcctact aaaagtcatt tgttacctaa
3481 acttatgtgc ttaacaggca atgcttctca gaccacaaag cagaaagaag aagaaaagct
3541 cctgactaaa tcagggctgg gcttagacag agttgatctg tagaatatct ttaaaggaga
3601 gatgtcaact ttctgcacta ttcccagcct ctgctcctcc ctgtctaccc tctcccctcc
3661 ctctctccct ccacttcacc ccacaatctt gaaaaacttc ctttctcttc tgtgaacatc
3721 attggccaga tccattttca gtggtctgga tttcttttta ttttcttttc aacttgaaag
3781 aaactggaca ttaggccact atgtgttgtt actgccacta gtgttcaagt gcctcttgtt
3841 ttcccagaga tttcctgggt ctgccagagg cccagacagg ctcactcaag ctctttaact
3901 gaaaagcaac aagccactcc aggacaaggt tcaaaatggt tacaacagcc tctacctgtc
3961 gccccaggga gaaaggggta gtgatacaag tctcatagcc agagatggtt ttccactcct
4021 tctagatatt cccaaaaaga ggctgagaca ggaggttatt ttcaatttta ttttggaatt
4081 aaatactttt ttccctttat tactgttgta gtccctcact tggatatacc tctgttttca
4141 cgatagaaat aagggaggtc tagagcttct attccttggc cattgtcaac ggagagctgg
4201 ccaagtcttc acaaaccctt gcaacattgc ctgaagttta tggaataaga tgtattctca
4261 ctcccttgat ctcaagggcg taactctgga agcacagctt gactacacgt catttttacc
4321 aatgattttc aggtgacctg ggctaagtca tttaaactgg gtctttataa aagtaaaagg
4381 ccaacattta attattttgc aaagcaacct aagagctaaa gatgtaattt ttcttgcaat
4441 tgtaaatctt ttgtgtctcc tgaagacttc ccttaaaatt agctctgagt gaaaaatcaa
4501 aagagacaaa agacatcttc gaatccatat ttcaagcctg gtagaattgg cttttctagc
4561 agaacctttc caaaagtttt atattgagat tcataacaac accaagaatt gattttgtag
4621 ccaacattca ttcaatactg ttatatcaga ggagtaggag agaggaaaca tttgacttat
4681 ctggaaaagc aaaatgtact taagaataag aataacatgg tccattcacc tttatgttat
4741 agatatgtct ttgtgtaaat catttgtttt gagttttcaa agaatagccc attgttcatt
4801 cttgtgctgt acaatgacca ctgttattgt tactttgact tttcagagca cacccttcct
4861 ctggtttttg tatatttatt gatggatcaa taataatgag gaaagcatga tatgtatatt
4921 gctgagttga aagcacttat tggaaaatat taaaaggcta acattaaaag actaaaggaa
4981 acagaaaaaa aaaaaaaaaa a
```

NM_001001391 transcript variant 4

```
   1 gagaagaaag ccagtgcgtc tctgggcgca ggggccagtg gggctcggag gcacaggcac
  61 cccgcgacac tccaggttcc ccgacccacg tccctggcag ccccgattat ttacagcctc
 121 agcagagcac ggggcggggg cagaggggcc cgcccgggag ggctgctact tcttaaaacc
 181 tctgcgggct gcttagtcac agccccccct gcttgggtgt gtccttcgct cgctccctcc
 241 ctccgtctta ggtcactgtt ttcaacctcg aataaaaact gcagccaact tccgaggcag
 301 cctcattgcc cagcggaccc cagcctctgc caggttcggt ccgccatcct cgtcccgtcc
 361 tccgccggcc cctgccccgc gcccagggat cctccagctc ctttcgcccg cgccctccgt
 421 tcgctccgga caccatggac aagttttggt ggcacgcagc ctggggactc tgcctcgtgc
 481 cgctgagcct ggcgcagatc gatttgaata taacctgccg ctttgcaggt gtattccacg
 541 tggagaaaaa tggtcgctac agcatctctc ggacggaggc cgctgacctc tgcaaggctt
 601 tcaatagcac cttgcccaca atggcccaga tggagaaagc tctgagcatc ggatttgaga
 661 cctgcaggta tgggttcata gaagggcacg tggtgattcc ccggatccac cccaactcca
 721 tctgtgcagc aaacaacaca ggggtgtaca tcctcacatc caacacctcc cagtatgaca
 781 catattgctt caatgcttca gctccacctg aagaagattg tacatcagtc acagacctgc
 841 ccaatgcctt tgatggacca attaccataa ctattgttaa ccgtgatggc acccgctatg
 901 tccagaaagg agaatacaga acgaatcctg aagacatcta ccccagcaac cctactgatg
 961 atgacgtgag cagcggctcc tccagtgaaa ggagcagcac ttcaggaggt tacatctttt
1021 acacctttc tactgtacac cccatcccag acgaagacag tccctggatc accgacagca
1081 cagacagaat ccctgctacc agagaccaag acacattcca ccccagtggg gggtcccata
1141 ccactcatgg atctgaatca gatggacact cacatgggag tcaagaaggt ggagcaaaca
1201 caacctctgg tcctataagg acaccccaaa ttccagaatg gctgatcatc ttggcatccc
1261 tcttggcctt ggctttgatt cttgcagttt gcattgcagt caacagtcga agaaggtgtg
1321 ggcagaagaa aaagctagtg atcaacagtg gcaatggagc tgtggaggac agaaagccaa
1381 gtggactcaa cggagaggcc agcaagtctc aggaaatggt gcatttggtg aacaaggagt
1441 cgtcagaaac tccagaccag tttatgacag ctgatgagac aaggaacctg cagaatgtgg
1501 acatgaagat tggggtgtaa cacctacacc attatcttgg aaagaaacaa ccgttggaaa
1561 cataaccatt acagggagct gggacactta acagatgcaa tgtgctactg attgtttcat
```

Figure 21 (Continued)

```
1621 tgcgaatctt ttttagcata aaatttttcta ctctttttgt tttttgtgtt ttgttcttta
1681 aagtcaggtc caatttgtaa aaacagcatt gctttctgaa attagggccc aattaataat
1741 cagcaagaat ttgatcgttc cagttcccac ttggaggcct ttcatccctc gggtgtgcta
1801 tggatggctt ctaacaaaaa ctacacatat gtattcctga tcgccaacct ttcccccacc
1861 agctaaggac atttcccagg gttaataggg cctggtccct gggaggaaat ttgaatgggt
1921 ccattttgcc cttccatagc ctaatccctg ggcattgctt tccactgagg ttgggggttg
1981 gggtgtacta gttacacatc ttcaacagac cccctctaga aatttttcag atgcttctgg
2041 gagacaccca aagggtgaag ctatttatct gtagtaaact atttatctgt gtttttgaaa
2101 tattaaaccc tggatcagtc ctttgatcag tataattttt taaagttact ttgtcagagg
2161 cacaaaaggg tttaaactga ttcataataa atatctgtac ttcttcgatc ttcacctttt
2221 gtgctgtgat tcttcagttt ctaaaccagc actgtctggg tccctacaat gtatcaggaa
2281 gagctgagaa tggtaaggag actcttctaa gtcttcatct cagagaccct gagttcccac
2341 tcagacccac tcagccaaat ctcatggaag accaaggagg gcagcactgt ttttgttttt
2401 tgtttttttgt ttttttttttt tgacactgtc caaaggtttt ccatcctgtc ctggaatcag
2461 agttggaagc tgaggagctt cagcctcttt tatggtttaa tggccacctg ttctctcctg
2521 tgaaaggctt tgcaaagtca cattaagttt gcatgacctg ttatccctgg ggccctattt
2581 catagaggct ggccctatta gtgatttcca aaaacaatat ggaagtgcct tttgatgtct
2641 tacaataaga gaagaagcca atggaaatga aagagattgg caaaggggaa ggatgatgcc
2701 atgtagatcc tgtttgacat ttttatggct gtatttgtaa acttaaacac accagtgtct
2761 gttcttgatg cagttgctat ttaggatgag ttaagtgcct ggggagtccc tcaaaaggtt
2821 aaagggattc ccatcattgg aatcttatca ccagataggc aagtttatga ccaaacaaga
2881 gagtactggc tttatcctct aacctcatat tttctcccac ttggcaagtc ctttgtggca
2941 tttattcatc agtcagggtg tccgattggt cctagaactt ccaaaggctg cttgtcatag
3001 aagccattgc atctataaag caacggctcc tgttaaatgg tatctccttt ctgaggctcc
3061 tactaaaagt catttgttac ctaaacttat gtgcttaaca ggcaatgctt ctcagaccac
3121 aaagcagaaa gaagaagaaa agctcctgac taaatcaggg ctgggcttag acagagttga
3181 tctgtagaat atctttaaag gagagatgtc aactttctgc actattccca gcctctgctc
3241 ctccctgtct accctctccc ctccctctct ccctccactt caccccacaa tcttgaaaaa
3301 cttccttttct cttctgtgaa catcattggc cagatccatt ttcagtggtc tggatttctt
3361 tttatttttct tttcaacttg aaagaaactg gacattaggc cactatgtgt tgttactgcc
3421 actagtgttc aagtgcctct tgttttccca gagatttcct gggtctgcca gaggcccaga
3481 caggctcact caagctcttt aactgaaaag caacaagcca ctccaggaca aggttcaaaa
3541 tggttacaac agcctctacc tgtcgcccca gggagaaagg ggtagtgata caagtctcat
3601 agccagagat ggttttccac tccttctaga tattcccaaa aagaggctga gacaggaggt
3661 tattttcaat tttatttttgg aattaaatac ttttttccct ttattactgt tgtagtccct
3721 cacttggata tacctctgtt ttcacgatag aaataaggga ggtctagagc ttctattcct
3781 tggccattgt caacggagag ctggccaagt cttcacaaac ccttgcaaca ttgcctgaag
3841 tttatggaat aagatgtatt ctcactccct tgatctcaag ggcgtaactc tggaagcaca
3901 gcttgactac acgtcatttt taccaatgat tttcaggtga cctgggctaa gtcatttaaa
3961 ctgggtcttt ataaaagtaa aaggccaaca tttaattatt ttgcaaagca acctaagagc
4021 taaagatgta atttttcttg caattgtaaa tcttttgtgt ctcctgaaga cttcccttaa
4081 aattagctct gagtgaaaaa tcaaaagaga caaaagacat cttcgaatcc atatttcaag
4141 cctggtagaa ttggcttttc tagcagaacc tttccaaaag ttttatattg agattcataa
4201 caacaccaag aattgatttt gtagccaaca ttcattcaat actgttatat cagaggagta
4261 ggagagagga aacatttgac ttatctggaa aagcaaaatg tacttaagaa taagaataac
4321 atggtccatt caccttttatg ttatagatat gtctttgtgt aaatcatttg ttttgagttt
4381 tcaaagaata gcccattgtt cattcttgtg ctgtacaatg accactgtta ttgttacttt
4441 gacttttcag agcacaccct tcctctggtt tttgtatatt tattgatgga tcaataataa
4501 tgaggaaagc atgatatgta tattgctgag ttgaaagcac ttattggaaa atattaaaag
4561 gctaacatta aaagactaaa ggaaacagaa aaaaaaaaaa aaaaa
```

Figure 21 (Continued)

NM_001001392 transcript variant 5

```
   1 gagaagaaag ccagtgcgtc tctgggcgca ggggccagtg gggctcggag gcacaggcac
  61 cccgcgacac tccaggttcc ccgacccacg tccctggcag ccccgattat ttacagcctc
 121 agcagagcac ggggcggggg cagaggggcc cgcccgggag ggctgctact tcttaaaacc
 181 tctgcgggct gcttagtcac agccccccтt gcttgggtgt gtccttcgct cgctccctcc
 241 ctccgtctta ggtcactgtt ttcaacctcg aataaaaact gcagccaact tccgaggcag
 301 cctcattgcc cagcggaccc cagcctctgc caggttcggt ccgccatcct cgtcccgtcc
 361 tccgccggcc cctgccccgc gcccagggat cctccagctc ctttcgcccg cgccctccgt
 421 tcgctccgga caccatggac aagttttggt ggcacgcagc ctggggactc tgcctcgtgc
 481 cgctgagcct ggcgcagatc gatttgaata taacctgccg ctttgcaggt gtattccacg
 541 tggagaaaaa tggtcgctac agcatctctc ggacggaggc cgctgacctc tgcaaggctt
 601 tcaatagcac cttgcccaca atggcccaga tggagaaagc tctgagcatc ggatttgaga
 661 cctgcagttt gcattgcagt caacagtcga agaaggtgtg ggcagaagaa aaagctagtg
 721 atcaacagtg gcaatggagc tgtggaggac agaaagccaa gtggactcaa cggagaggcc
 781 agcaagtctc aggaaatggt gcatttggtg aacaaggagt cgtcagaaac tccagaccag
 841 tttatgacag ctgatgagac aaggaacctg cagaatgtgg acatgaagat tggggtgtaa
 901 cacctacacc attatcttgg aaagaaacaa ccgttggaaa cataaccatt acagggagct
 961 gggacactta acagatgcaa tgtgctactg attgtttcat tgcgaatctt ttttagcata
1021 aaatttтcta ctcttttttgt tttttgtgtt ttgttcttta aagtcaggtc caatttgtaa
1081 aaacagcatt gctttctgaa attagggccc aattaataat cagcaagaat ttgatcgttc
1141 cagttcccac ttggaggcct ttcatccctc gggtgtgcta tggatggctt ctaacaaaaa
1201 ctacacatat gtattcctga tcgccaacct ttcccccacc agctaaggac atttcccagg
1261 gttaataggg cctggtccct gggaggaaat ttgaatgggt ccattttgcc cttccatagc
1321 ctaatccctg ggcattgctt tccactgagg ttgggggttg gggtgtacta gttacacatc
1381 ttcaacagac cccctctaga aatttttcag atgcttctgg gagacaccca aagggtgaag
1441 ctatttatct gtagtaaact atttatctgt gtttttgaaa tattaaaccc tggatcagtc
1501 ctttgatcag tataattttt taaagttact ttgtcagagg cacaaaaggg tttaaactga
1561 ttcataataa atatctgtac ttcttcgatc ttcacctttt gtgctgtgat tcttcagttt
1621 ctaaaccagc actgtctggg tccctacaat gtatcaggaa gagctgagaa tggtaaggag
1681 actcttctaa gtcttcatct cagagaccct gagttcccac tcagacccac tcagccaaat
1741 ctcatggaag accaaggagg gcagcactgt ttttgttttt tgtttttttgt ttttttttt
1801 tgacactgtc caaaggtttt ccatcctgtc ctggaatcag agttggaagc tgaggagctt
1861 cagcctcttt tatggtttaa tggccacctg ttctctcctg tgaaaggctt tgcaaagtca
1921 cattaagttt gcatgacctg ttatccctgg ggccctattt catagaggct ggccctatta
1981 gtgatttcca aaaacaatat ggaagtgcct tttgatgtct tacaataaga gaagaagcca
2041 atggaaatga aagagattgg caaaggggaa ggatgatgcc atgtagatcc tgtttgacat
2101 ttttatggct gtatttgtaa acttaaacac accagtgtct gttcttgatg cagttgctat
2161 ttaggatgag ttaagtgcct ggggagtccc tcaaaaggtt aaagggattc ccatcattgg
2221 aatcttatca ccagataggc aagtttatga ccaaacaaga gagtactggc tttatcctct
2281 aacctcatat tttctcccac ttggcaagtc ctttgtggca tttattcatc agtcagggtg
2341 tccgattggt cctagaactt ccaaaggctg cttgtcatag aagccattgc atctataaag
2401 caacggctcc tgttaaatgg tatctccttt ctgaggctcc tactaaaagt catttgttac
2461 ctaaacttat gtgcttaaca ggcaatgctt ctcagaccac aaagcagaaa gaagaagaaa
2521 agctcctgac taaatcaggg ctgggcttag acagagttga tctgtagaat atctttaaag
2581 gagagatgtc aactttctgc actattccca gcctctgctc ctccctgtct accctctccc
2641 ctccctctct ccctccactt caccccacaa tcttgaaaaa cttcctttct cttctgtgaa
2701 catcattggc cagatccatt ttcagtggtc tggatttctt tttattttct tttcaacttg
2761 aaagaaactg gacattaggc cactatgtgt tgttactgcc actagtgttc aagtgcctct
2821 tgttttccca gagatttcct gggtctgcca gaggcccaga caggctcact caagctcttt
2881 aactgaaaag caacaagcca ctccaggaca aggttcaaaa tggttacaac agcctctacc
2941 tgtcgcccca gggagaaagg ggtagtgata caagtctcat agccagagat ggttttccac
3001 tccttctaga tattcccaaa aagaggctga gacaggaggt tattttcaat tttattttgg
3061 aattaaatac ttttttccct ttattactgt tgtagtccct cacttggata tacctctgtt
3121 ttcacgatag aaataaggga ggtctagagc ttctattcct tggccattgt caacggagag
3181 ctggccaagt cttcacaaac ccttgcaaca ttgcctgaag tttatggaat aagatgtatt
3241 ctcactccct tgatctcaag ggcgtaactc tggaagcaca gcttgactac acgtcatttt
3301 taccaatgat tttcaggtga cctgggctaa gtcatttaaa ctgggtcttt ataaaagtaa
3361 aaggccaaca tttaattatt ttgcaaagca acctaagagc taaagatgta atttttcttg
```

Figure 21 (continued)

```
3421 caattgtaaa tcttttgtgt ctcctgaaga cttcccttaa aattagctct gagtgaaaaa
3481 tcaaaagaga caaaagacat cttcgaatcc atatttcaag cctggtagaa ttggcttttc
3541 tagcagaacc tttccaaaag ttttatattg agattcataa caacaccaag aattgatttt
3601 gtagccaaca ttcattcaat actgttatat cagaggagta ggagagagga aacatttgac
3661 ttatctggaa aagcaaaatg tacttaagaa taagaataac atggtccatt cacctttatg
3721 ttatagatat gtctttgtgt aaatcatttg ttttgagttt tcaaagaata gcccattgtt
3781 cattcttgtg ctgtacaatg accactgtta ttgttacttt gacttttcag agcacaccct
3841 tcctctggtt tttgtatatt tattgatgga tcaataataa tgaggaaagc atgatatgta
3901 tattgctgag ttgaaagcac ttattggaaa atattaaaag gctaacatta aaagactaaa
3961 ggaaacagaa aaaaaaaaaa aaaaa
```

X62739 Isoform identified in tumour cells

```
   1 gtacgtcttc aaataccatc tcagcaggct gggagccaaa tgaagaaaat gaagatgaaa
  61 gagacagaca cctcagtttt tctggatcag gcattgatga tgatgaagat tttatctcca
 121 gcaccatttc aaccacacca cgggcctttg accacacaaa acagaaccag gactggaccc
 181 agtggaaccc aagccattca aatccggaag tgctacttca gacaaccaca aggatgactg
 241 atgtagacag aaatggcacc actgcttatg aaggaaactg gaacccagaa gcacaccctc
 301 ccctcattca ccatgagcat catgaggaag aagagacccc acattctaca agcacaatcc
 361 aggcaactcc tagtagtaca acggaagaaa cagctaccca gaaggaacag tggtttggca
 421 acagatggca tgagggatat cgccaaacac ccagagaaga ctcccattcg acaacaggga
 481 cagctgcagc ctcagctcat accagccatc caatgcaagg aaggacaaca ccaagcccag
 541 aggacagttc ctggactgat ttcttcaacc caatctcaca ccccatggga cgaggtcatc
 601 aagcaggaag aaggatggat atggactcca gtcatagtac aacgcttcag cctactgcaa
 661 atccaaacac aggtttggtg gaagatttgg acaggacagg acctctttca atgacaacgc
 721 agcagagtaa ttctcagagc ttctctacat cacatgaagg cttggaagaa gataaagacc
 781 atccaacaac ttctactctg acatcaagca ataggaatga tgtcacaggt ggaagaagag
 841 acccaaatca ttctgaaggc tcaactactt tactggaagg ttatacctct cattacccac
 901 acacgaagga aagcaggacc ttcatcccag tgacctcagc taagactggg tcctttggag
 961 ttactgcagt tactgttgga gattccaact ctaatgtcaa tcgttcctta tcag
```

Figure 21 (continued)

NP_000601 isoform 1 precursor

```
   1 mdkfwwhaaw glclvplsla qidlnitcrf agvfhveknq rysisrteaa dlckafnstl
  61 ptmaqmekal sigfetcryg fieghvvipr ihpnsicaan ntgvyiltsn tsqydtycfn
 121 asappeedct svtdlpnafd gpititivnr dgtryvqkge yrtnpediyp snptdddvss
 181 gsssserssts ggyifytfst vhpipdedsp witdstdrip attlmstsat atetatkrqe
 241 twdwfswlfl psesknhlht ttqmagtssn tisagwepne enederdrhl sfsgsgiddd
 301 edfisstist tprafdhtkq nqdwtqwnps hsnpevllqt ttrmtdvdrn gttayegnwn
 361 peahpplihh ehheeeetph ststiqatps stteetatqk eqwfgnrwhe gyrqtpkeds
 421 hsttgtaaas ahtshpmqgr ttpspedssw tdffnpishp mgrghqagrr mdmdsshsit
 481 lqptanpntg lvedldrtgp lsmttqqsns qsfstshegl eedkdhptts tltssnrndv
 541 tggrrdpnhs egsttllegy tshyphtkes rtfipvtsak tgsfgvtavt vgdsnsnvnr
 601 slsgdqdtfh psggshtthg sesdghshgs qegganttsg pirtpqipew liilasllal
 661 alilavciav nsrrrcgqkk klvinsgnga vedrkpsgln geasksqemv hlvnkesset
 721 pdqfmtadet rnlqnvdmki gv
```

NP_001001389 isoform 2 precursor

```
   1 mdkfwwhaaw glclvplsla qidlnitcrf agvfhveknq rysisrteaa dlckafnstl
  61 ptmaqmekal sigfetcryg fieghvvipr ihpnsicaan ntgvyiltsn tsqydtycfn
 121 asappeedct svtdlpnafd gpititivnr dgtryvqkge yrtnpediyp snptdddvss
 181 gsssserssts ggyifytfst vhpipdedsp witdstdrip atstssntis agwepneene
 241 derdrhlsfs gsgidddedf isstisttpr afdhtkqnqd wtqwnpshsn pevllqtttr
 301 mtdvdrngtt ayegnwnpea hpplihhehh eeeetphsts tiqatpsstt eetatqkeqw
 361 fgnrwhegyr qtpkedshst tgtaaasaht shpmqgrttp spedsswtdf fnpishpmgr
 421 ghqagrrmdm dsshsitlqp tanpntglve dldrtgplsm ttqqsnsqsf stshegleed
 481 kdhpttstlt ssnrndvtgg rrdpnhsegs ttllegytsh yphtkesrtf ipvtsaktgs
 541 fgvtavtvgd snsnvnrsls gdqdtfhpsg gshtthgses dghshgsqeg ganttsgpir
 601 tpqipewlii lasllalali lavciavnsr rrcgqkkklv insgngaved rkpsglngea
 661 sksqemvhlv nkessetpdq fmtadetrnl qnvdmkigv
```

NP_001001390 isoform 3 precursor

```
   1 mdkfwwhaaw glclvplsla qidlnitcrf agvfhveknq rysisrteaa dlckafnstl
  61 ptmaqmekal sigfetcryg fieghvvipr ihpnsicaan ntgvyiltsn tsqydtycfn
 121 asappeedct svtdlpnafd gpititivnr dgtryvqkge yrtnpediyp snptdddvss
 181 gsssserssts ggyifytfst vhpipdedsp witdstdrip atnmdsshsi tlqptanpnt
 241 glvedldrtg plsmttqqsn sqsfstsheg leedkdhptt stltssnrnd vtggrrdpnh
 301 segsttlleg ytshyphtke srtfipvtsa ktgsfgvtav tvgdsnsnvn rslsgdqdtf
 361 hpsggshtth gsesdghshg sqeggantts gpirtpqipe wliilaslla lalilavcia
 421 vnsrrrcgqk kklvinsgng avedrkpsgl ngeasksqem vhlvnkesse tpdqfmtade
 481 trnlqnvdmk igv
```

Figure 21 (continued)

NP_001001391 isoform 4 precursor

```
  1 mdkfwwhaaw glclvplsla qidlnitcrf agvfhveknq rysisrteaa dlckafnstl
 61 ptmaqmekal sigfetcryg fieghvvipr ihpnsicaan ntgvyiltsn tsqydtycfn
121 asappeedct svtdlpnafd gpititivnr dgtryvqkge yrtnpediyp snptdddvss
181 gsssserssts ggyifytfst vhpipdedsp witdstdrip atrdqdtfhp sggshtthgs
241 esdghshgsq egganttsgp irtpqipewl iilasllala lilavciavn srrrcgqkkk
301 lvinsgngav edrkpsglng easksqemvh lvnkessetp dqfmtadetr nlqnvdmkig
361 v
```

NP_001001392 isoform 5 precursor

```
  1 mdkfwwhaaw glclvplsla qidlnitcrf agvfhveknq rysisrteaa dlckafnstl
 61 ptmaqmekal sigfetcslh csqqskkvwa eekasdqqwq wscgggkakw tqrrgqqvsg
121 ngafgeqgvv rnsrpvyds
```

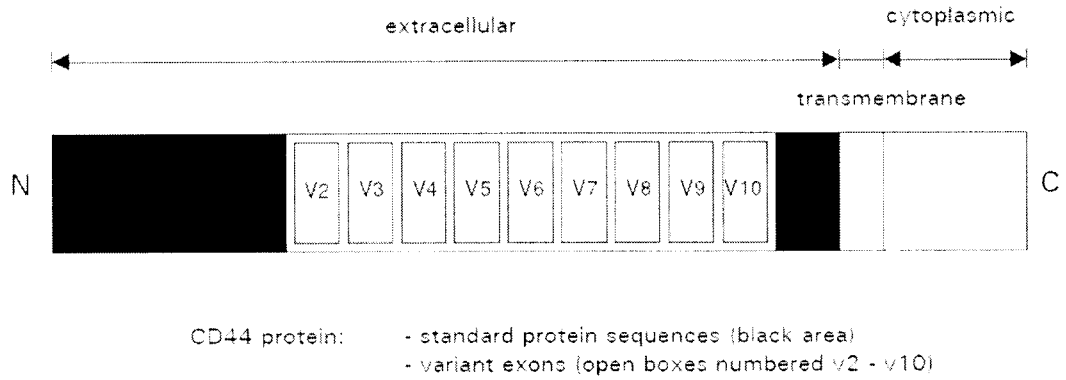
CAA44602 Isoform identified in tumour cells

```
  1 tssntisagw epneeneder drhlsfsgsg idddedfiss tisttprafd htkqnqdwtq
 61 wnpshsnpev llqtttrmtd vdrngttaye gnwnpeahpp lihhehheee etphststiq
121 atpsstteet atqkeqwfgn rwhegyrqtp redshsttgt aaasahtshp mqgrttpspe
181 dsswtdffnp ishpmgrghq agrrmdmdss hsttlqptan pntglvedld rtgplsmttq
241 qsnsqsfsts hegleedkdh pttstltssn rndvtggrrd pnhsegsttl legytshyph
301 tkesrtfipv tsaktgsfgv tavtvgdsns nvnrsls
```

Figure 21 (continued)

A

The sCD44std ELISA detects all circulating CD44 isoforms comprising the standard protein sequences (black area).



B

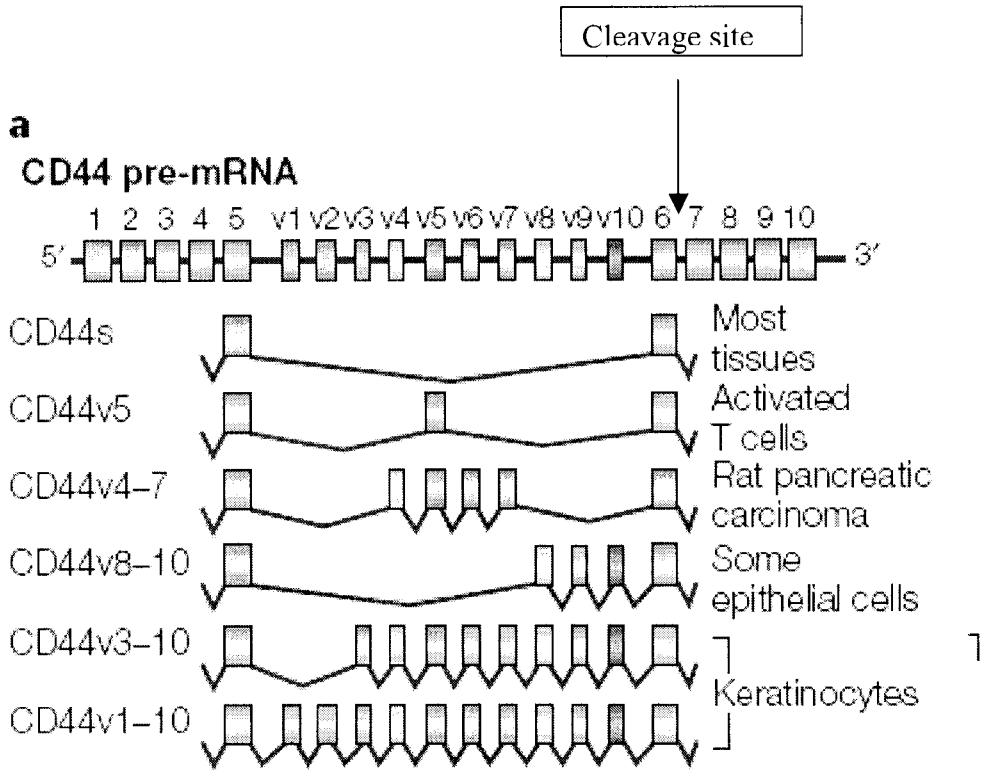


Figure 22

C

XLNITCRFAGVFHVEKNGRYSISRTEAADLCKAFNSTLPTMAQMEKALSIGFETCRYGFIEG
HVVIPRIHPNSICAANNTGVYILTSNTSQYDTYCFNASAPPEEDCTSVTDLPNAFDGPITIT
IVNRDGTRYVQKGEYRTNPEDIYPSNPTDDDVSSGSSSERSSTSGGYIFYTFSTVHPIPDED
SPWITDSTDRIPATTLMSTSATATETATKRQETWDWFSWLFLPSESKNHLHTTTQMAGTSSN
TISAGWEPNEENEDERDRHLSFSGSGIDDDEDFISSTISTTPRAFDHTKQNQDWTQWNPSHS
NPEVLLQTTTRMTDVDRNGTTAYEGNWNPEAHPPLIHHEHHEEEETPHSTSTIQATPSSTTE
ETATQKEQWFGNRWHEGYRQTPKEDSHSTTGTAAASAHTSHPMQGRTTPSPEDSSWTDFFNP
ISHPMGRGHQAGRRMDMDSSHSITLQPTANPNTGLVEDLDRTGPLSMTTQQSNSQSFSTSHE
GLEEDKDHPTTSTLTSSNRNDVTGGRRDPNHSEGSTTLLEGYTSHYPHTKESRTFIPVTSAK
TGSFGVTAVTVGDSNSNVNRSLSGDQDTFHPSGGSHTTHGSESDGHSHGSQEGGANTTSGPI
RTPQIP**E**WLIILASLLALALILAVCIAVNSRRRCGQKKKLVINSGNGAVEDRKPSGLNGEAS
KSQEMVHLVNKESSETPDQFMTADETRNLQNVDMKIGV

Cleavage site

Figure 22 (Continued)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000595

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

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

1. [X] Claim Nos. : 31-34
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 31-34 are directed to a method of medical treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search.

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

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

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

see extra sheet

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

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

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

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

Remark on Protest

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

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

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000595

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***G01N 33/53*** (2006.01) , ***A61K 33/04*** (2006.01) , ***A61K 45/00*** (2006.01) , ***A61P 19/08*** (2006.01) , ***C12Q 1/68*** (2006.01) , ***G01N 33/543*** (2006.01) (more IPCs on the last page)

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)

G01N 33/53 (2006.01) , ***A61K 33/04*** (2006.01) , ***A61K 45/00*** (2006.01) , ***A61P 19/08*** (2006.01) , ***C12Q 1/68*** (2006.01) , ***G01N 33/543*** (2006.01), ***G01N 33/68*** (2006.01) , ***C07K 14/52*** (2006.01) , ***C07K 14/705*** (2006.01), ***C12Q 1/00*** (2006.01)

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

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

CPD, Derwent, Scopus, PubMed, Google search terms: scoliosis, osteopontin, bone sialoprotein, secreted phosphoprotein, early T-lymphocyte activation, Eta-1, minopontin, CD44, soluble

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOR, G. et al. 'Serum protein markers for early detection of ovarian cancer' PNAS (2005) vol. 102, no. 21, pages 7677-7682, see especially p. 7679.	48
A	US 2005/0130250 A1 (MOREAU, A.) 16 Jun 2005 (16-06-2005) *Whole document*	1-49
A	BERTRAM, H. et al. 'Accelerated intervertebral disc degeneration in scoliosis versus physiological ageing develops against a background of enhanced anabolic gene expression' BIOCHEM. BIOPHYS. RES. COMM. (2006) vol. 342, pages 963-972, see entire document.	1-49

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 July 2008 (03-07-2008)	18 July 2008 (18-07-2008)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Kathryn Moore 819- 934-9088

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2008/000595

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2005130250	16-06-2005	AT385574T T	15-02-2008
		AU2003208204 A1	09-09-2003
		CA2373854 A1	28-08-2003
		DE60318987D D1	20-03-2008
		DK1478928T T3	19-05-2008
		EP1478928 A1	24-11-2004
		ES2298498T T3	16-05-2008
		WO03073102 A1	04-09-2003

Continuation of Box No. III

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-27, 48, 49 (completely); 35-37 (partially) are directed to methods for determining risk of developing a scoliosis comprising measuring osteopontin expression.

Group B - Claims 28, 38, 41, 42 (completely); 30, 35-37, 47 (partially) are directed to a method of selecting as a potential candidate for reduction or prevention of scoliosis an inhibitor of osteopontin expression, and use of an osteopontin inhibitor for the treatment or prevention of scoliosis.

Group C - Claims 29, 40, 45, 46 (completely); 30, 35-37, 47 (partially) are directed to a method of selecting as a potential candidate for reduction or prevention of scoliosis a stimulator of sCD44 expression, and use of a sCD44 stimulator for the treatment or prevention of scoliosis.

Group D - Claims 39, 43, 44 (completely); 47 (partially) are directed to the use of a CD44 inhibitor for the treatment or prevention of scoliosis.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

G01N 33/68 (2006.01) , *C07K 14/52* (2006.01) , *C07K 14/705* (2006.01) , *C12Q 1/00* (2006.01)

专利名称(译)	确定脊柱侧凸风险的方法		
公开(公告)号	EP2132568A1	公开(公告)日	2009-12-16
申请号	EP2008733693	申请日	2008-03-31
申请(专利权)人(译)	褚SAINTE-JUSTINE		
当前申请(专利权)人(译)	褚SAINTE-JUSTINE		
[标]发明人	MOREAU ALAIN		
发明人	MOREAU, ALAIN		
IPC分类号	G01N33/53 A61K33/04 A61K45/00 A61P19/08 C12Q1/68 G01N33/543 G01N33/68 C07K14/52 C07K14/705 C12Q1/00 A23L33/00		
CPC分类号	A61F5/0102 A61K33/04 A61P19/08 A61P43/00 A23L33/30 G01N33/6872 G01N33/6893 G01N33/15 G01N33/53 A23V2002/00 G01N2800/108 G01N2800/50		
优先权	60/909408 2007-03-30 US 61/025571 2008-02-01 US		
其他公开文献	EP2132568A4 EP2132568B1		
外部链接	Espacenet		

摘要(译)

一种用于确定发展脊柱侧凸的风险的方法，包括随时间监测来自受试者的样品中的骨桥蛋白（OPN）表达;其中，受试者样品中随时间增加的OPN表达指示受试者有发展脊柱侧凸的风险。