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(54) **BIOMARKERS FOR MOTOR NEURON DISEASE**

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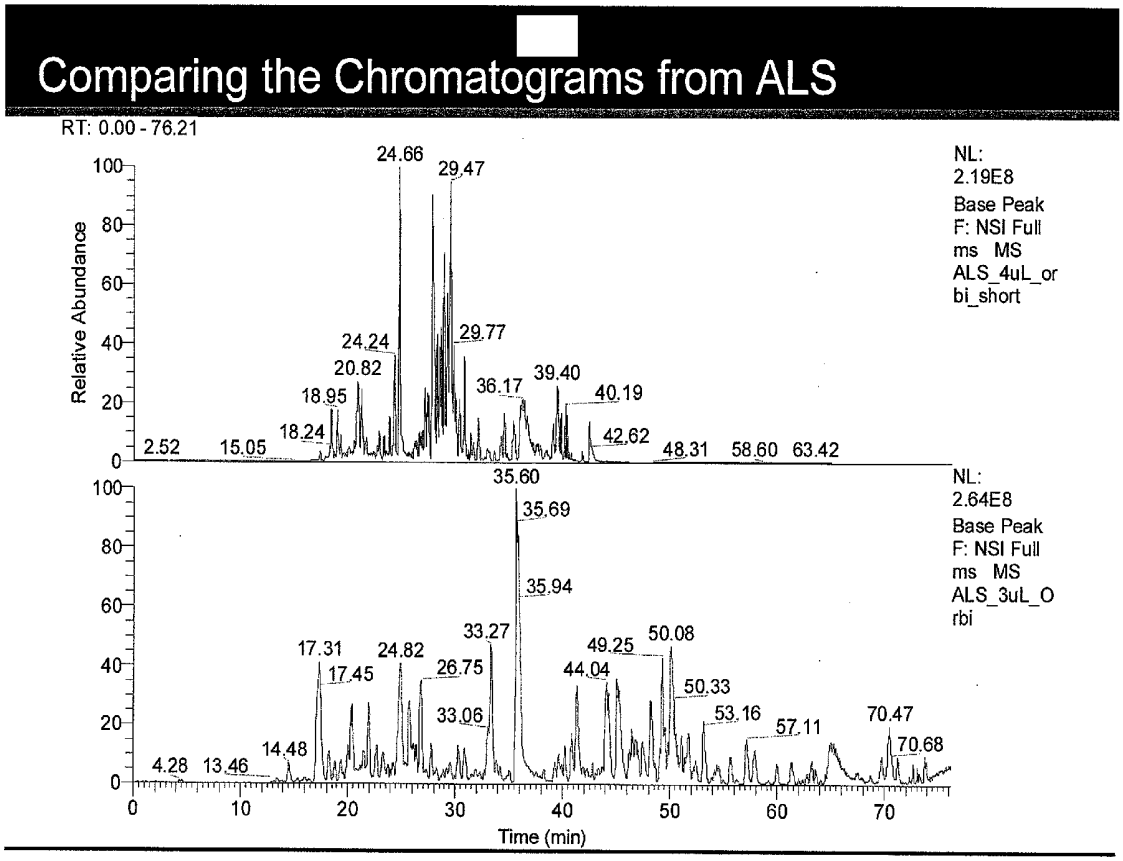
(52) **U.S. Cl.** **435/23**; 436/86; 435/29

(57) **ABSTRACT**

The invention provides methods of determining a diagnosis or prognosis of motor neuron disease in a mammal comprising determining the expression level of one or more proteins or polypeptides of the renin-angiotensin system in a sample taken from a subject. Similarly, aberrant post-translational modification of the proteins or polypeptides as compared to a negative control indicates a diagnosis of disease.



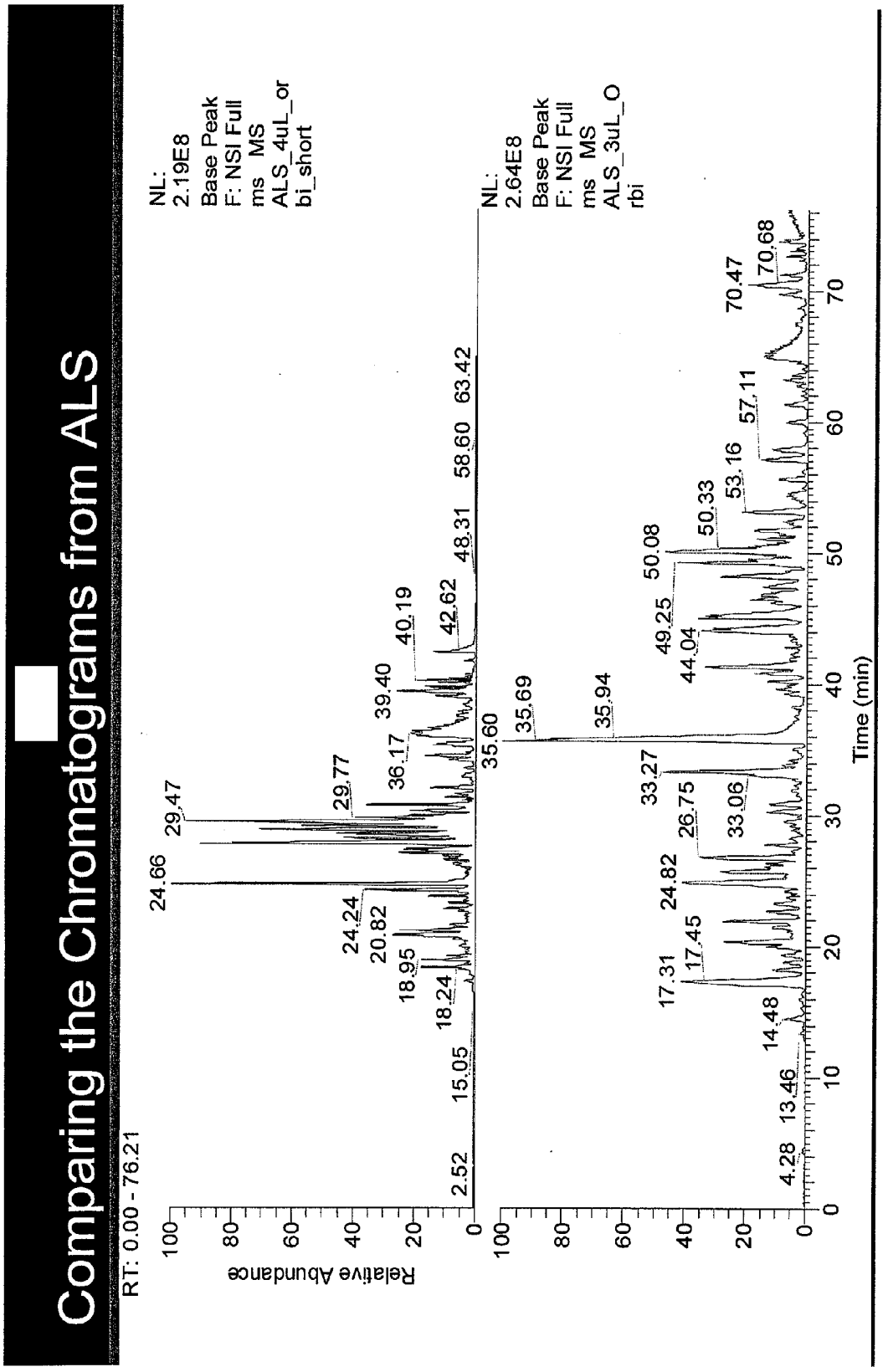

Comparing the Chromatograms from ALS
RT: 0.00 - 76.21
NL:
2.19E8
Base Peak
F: NSI Full
ms MS
ALS_4uL_or
bi_short
NL:
2.64E8
Base Peak
F: NSI Full
ms MS
ALS_3uL_O
rbi
Relative Abundance
Time (min)
2.52
4.28
13.46
14.48
15.05
17.31
17.45
18.24
18.95
20.82
24.24
24.66
24.82
26.75
29.47
29.77
33.06
33.27
35.60
35.69
35.94
36.17
39.40
40.19
42.62
44.04
48.31
49.25
50.08
50.33
53.16
57.11
58.60
63.42
70.47
70.68

FIGURE 1

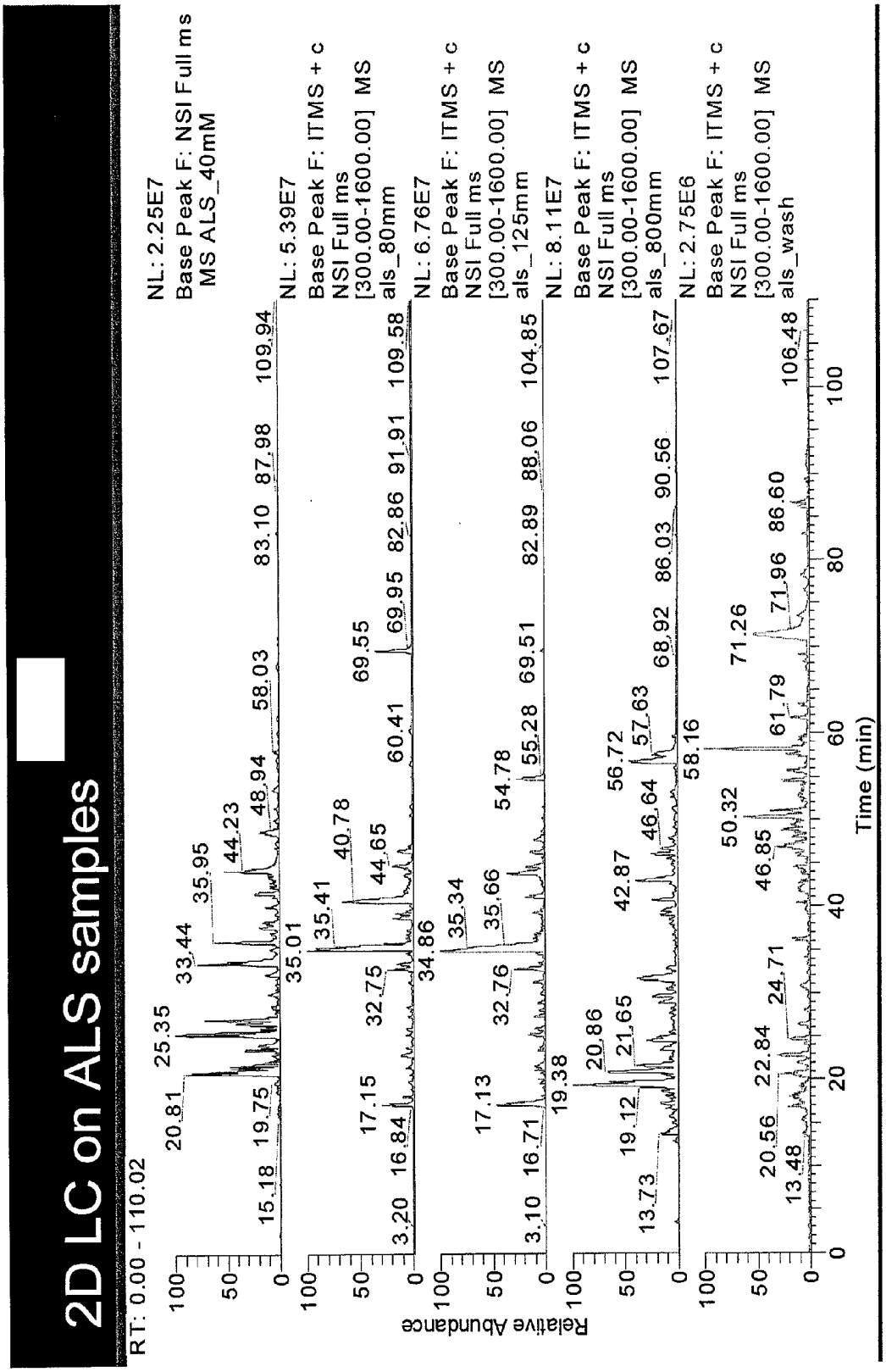

2D LC on ALS samples
RT: 0.00 - 110.02
NL: 2.25E7
Base Peak F: NSI Full ms
MS ALS_40mM
NL: 5.39E7
Base Peak F: ITMS + c
NSI Full ms
[300.00-1600.00] MS
als_80mm
NL: 6.76E7
Base Peak F: ITMS + c
NSI Full ms
[300.00-1600.00] MS
als_125mm
NL: 8.11E7
Base Peak F: ITMS + c
NSI Full ms
[300.00-1600.00] MS
als_800mm
NL: 2.75E6
Base Peak F: ITMS + c
NSI Full ms
[300.00-1600.00] MS
als_wash
Relative Abundance
Time (min)

FIGURE 2

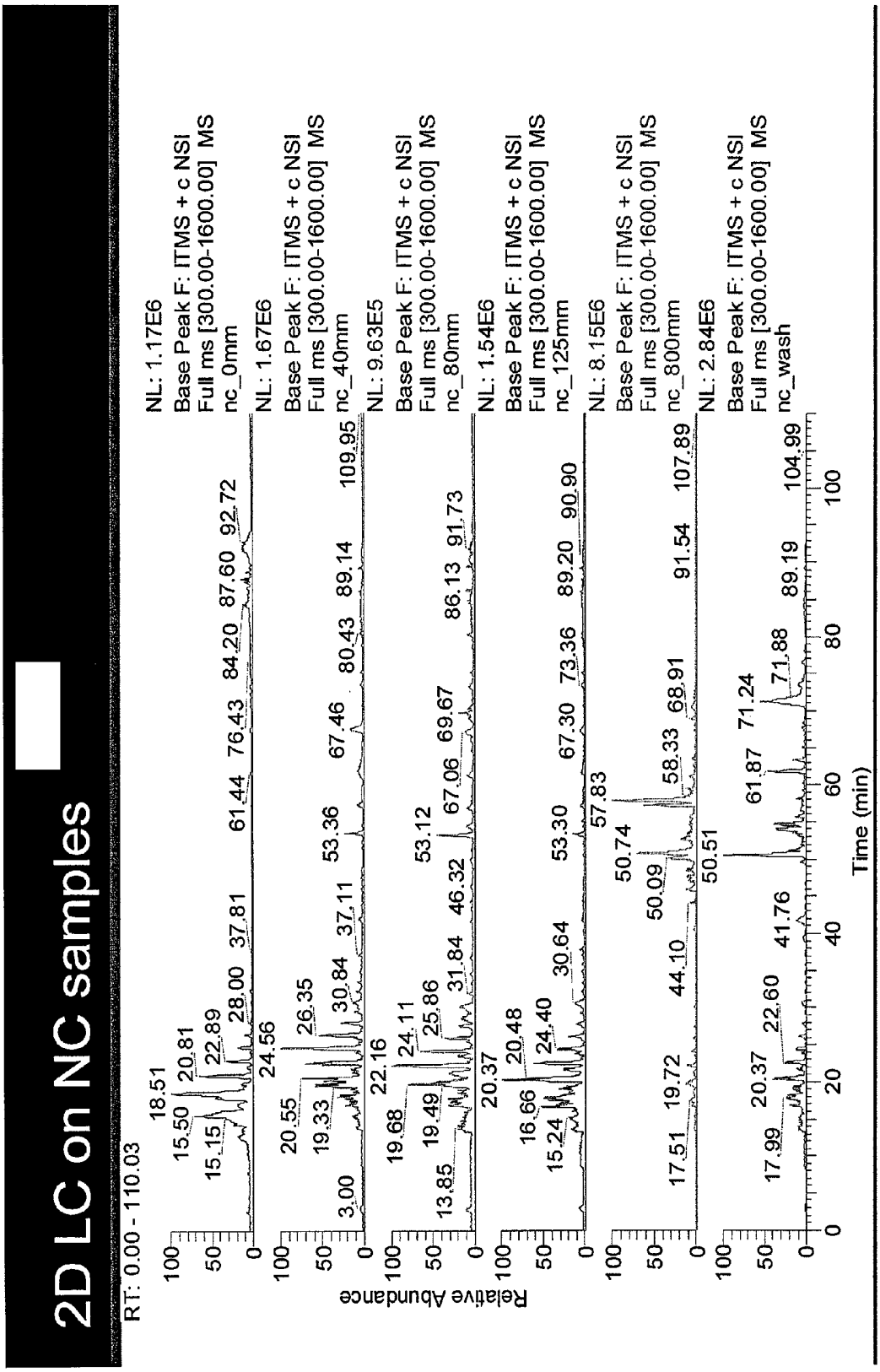

2D LC on NC samples
RT: 0.00 - 110.03
NL: 1.17E6
Base Peak F: ITMS + c NSI
Full ms [300.00-1600.00] MS
nc_0mm
NL: 1.67E6
Base Peak F: ITMS + c NSI
Full ms [300.00-1600.00] MS
nc_40mm
NL: 9.63E5
Base Peak F: ITMS + c NSI
Full ms [300.00-1600.00] MS
nc_80mm
NL: 1.54E6
Base Peak F: ITMS + c NSI
Full ms [300.00-1600.00] MS
nc_125mm
NL: 8.15E6
Base Peak F: ITMS + c NSI
Full ms [300.00-1600.00] MS
nc_800mm
NL: 2.84E6
Base Peak F: ITMS + c NSI
Full ms [300.00-1600.00] MS
nc_wash
Relative Abundance
Time (min)

FIGURE 3

BIOMARKERS FOR MOTOR NEURON DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority to U.S. Provisional Patent Application 60/971,709, filed on Sep. 12, 2007, the entire contents of which are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under Grant Number ES 013469 awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Motor neuron disease is a family of disorders characterized by progressive degeneration of upper and/or lower motor neurons. The most common form of adult-onset motor neuron disease is Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease. Other forms of motor neuron disease include primary lateral sclerosis, progressive muscular atrophy, pseudobulbar palsy, progressive bulbar palsy, lower motor neuron disease and spinal muscular atrophy.

[0004] There currently is one FDA approved drug for ALS (rilutek), but this only slows progression by a few months. Rapid diagnosis could potentially improve drug effects by introducing drug earlier in the disease course. However, diagnosis of motor neuron diseases such as ALS has been made clinically through neurological examination and exclusion of other disorders having similar manifestations. Diagnosis typically takes 6-12 months and multiple visits to specialists. Rapid diagnostic tests are not currently available for ALS and no markers of disease progression have been previously identified.

[0005] There remains a need for improved methods for identifying therapeutic targets of motor neuron disease, especially ALS, and improved methods of diagnosing the disease. Biomarkers of motor neuron disease could potentially assist measurements of drug efficacy in clinical trials and identify novel therapeutic targets for the generation of improved drug therapies.

BRIEF SUMMARY OF THE INVENTION

[0006] The invention provides methods of determining a diagnosis or prognosis of motor neuron disease in a mammal comprising determining the expression of one or more proteins of the renin-angiotensin system in a sample taken from a subject (e.g., a patient). Proteins in the pathway (or polypeptides from such proteins) can be quantified and compared to a negative control, wherein an aberrant quantity of one or more of the proteins or polypeptides indicates a diagnosis of motor neuron disease. Specifically, an increased quantity of certain proteins or polypeptides associated with the renin-angiotensin pathway indicates a diagnosis of disease, while a decreased quantity of certain proteins or polypeptides associated with the renin-angiotensin pathway indicates a diagnosis of disease. Similarly, aberrant post-translational modification of the proteins or polypeptides as compared to a negative control indicates a diagnosis of disease.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0007] FIG. **1** compares base peak chromatograms of an ALS sample and control sample generated from a full MS scan.

[0008] FIG. **2** presents exemplary base peak chromatograms of an ALS sample.

[0009] FIG. **3** presents exemplary base peak chromatograms of a control sample.

DETAILED DESCRIPTION OF THE INVENTION

[0010] In one aspect, the invention provides a method of diagnosing of motor neuron disease in a mammal. In one embodiment, the method comprises (1) providing a sample taken from a patient; (2) measuring or quantifying in the sample the amount of one or more proteins or polypeptides in the renin-angiotensin system (e.g., polypeptides comprising any of SEQ ID NOs: 1-63, polypeptides comprising a majority of contiguous amino acids of any of SEQ ID NOs: 1-63, or proteins associated therewith (see Table 1)); and (3) comparing the amount of the one or more protein or polypeptide sequences with a negative control. In accordance with this aspect of the invention, the presence of an aberrant quantity of one or more proteins or polypeptide sequences compared to the negative control indicates a diagnosis of motor neuron disease.

[0011] In some embodiments, one or more of the measured proteins or polypeptides is a protein or polypeptide for which increased levels relative to a negative control are associated with a diagnosis of motor neuron disease (e.g., SEQ ID NOs: 1-42, a polypeptide comprising a majority of contiguous amino acids from SEQ ID NOs: 1-42, or the proteins associated therewith (see, e.g., Table 1)). In other embodiments, one or more of the measured proteins or polypeptides is a protein or polypeptide for which decreased levels relative to a control are associated with a diagnosis of motor neuron disease (e.g., SEQ ID NOs: 43-63, a polypeptide comprising a majority of contiguous amino acids of SEQ ID NOs: 43-63, or a protein associated therewith (see Table 1)). In a preferred embodiment, two or more proteins or polypeptides are measured, with at least the first comprising a majority of contiguous amino acids of one of SEQ ID NOs: 1-42 and at least the second comprising a majority of contiguous amino acids of one of SEQ ID NOs: 43-63. For example the first can consist of or consist essentially of one of SEQ ID NOs: 1-42 and the second can consist of or consist essentially of one of SEQ ID NOs: 43-63.

[0012] In some embodiments, the diagnostic method further comprises detecting aberrant post-translational modification of the protein or polypeptide sequence(s) as compared to the control sample, wherein the presence of aberrant post-

translational modification indicates a diagnosis of motor neuron disease. Thus, for example, the invention provides a method of diagnosing motor neuron disease in a mammal comprising (1) providing a sample taken from a patient; (2) evaluating the sample for post-translational modification of one or more peptides of proteins in the renin-angiotensin pathway (e.g., polypeptides comprising any of SEQ ID NOs: 1-63, polypeptides comprising a majority of contiguous amino acids of any of SEQ ID NOs: 1-63, or proteins associated therewith (see Table 1)); and (3) comparing the post-translational modification of the peptides with a negative control. Aberrant post-translational modification of the peptides as compared to a negative control indicates a diagnosis of disease.

[0013] The post-translational modification can be any modification detectable by any method known to one of ordinary skill in the art, such as mass spectrometry proteomics. For example, the post-translational modification can be phosphorylation, glycosylation, oxidation, or methylation. Exemplary protocols for determining levels of post-translational modifications are provided in Chi et al., *Proc Natl Acad Sci, USA* 104(7): 2193-2198 (2007); Burlingame, et al., *Methods* 36: 383-394 (2005); Webb D J, et al., *J Cell Sci* 118: 4925-4929 (2005).

[0014] The negative control for the diagnostic methods can be any suitable negative control known to one of ordinary skill in the art. In a preferred embodiment, the negative control is a sample taken from a non-diseased subject of the same species as the patient, i.e., a healthy subject. In other embodiments, the negative control is a profile of measurements understood to reflect expected levels of the peptides a non-diseased subject. In some embodiments, additional controls can be also be compared to the test sample, such as samples taken from diseased subjects or profiles of measurements understood to reflect peptide levels associated with motor neuron disease.

[0015] Without being bound by any particular theory, it is thought that SEQ ID NOs: 1-63 represent biomarkers for proteins relating to the renin-angiotensin system. Such proteins can be within the renin-angiotensin pathway, i.e., respond to renin-angiotensin activation, or can modulate the renin-angiotensin pathway. For example, the renin-angiotensin pathway is thought to be modulated by related systems, such as the plasma kallikrein/kinin system. See, e.g., Schmaier, Am. J. Physiol. Regul. Integr. Comp. Physiol. 285: 1-13 (2003). In the present invention, SEQ ID NOs: 1-63 are thought to represent one or more proteins such as angiotensin precursor, kallikrein 6 (isoform B and isoform A preproprotein), kininogen, clusterin, antihrombin III (Serpin C1), plasminogen, plasminogen activator/urokinase, pigment epithelial derived factor precursor (PEDF or Serpin F1), vitamin D binding protein precursor, Vitamin D binding protein variant, angiotensin preproprotein, insulin-like growth factor binding protein 6, insulin-like growth factor binding protein 7, coagulation factor II precursor, coagulation factor XII, or plasminogen activator (urokinase receptor isoform).

[0016] Exemplary biomarker sequences are provided in Table 1, indicating the protein represented by the polypeptide sequence. Periods (".") are putative sites for trypsin cleavage.

TABLE 1

Sequence	Increased/ Decreased in ALS (+/-)	Sequence	Increased/ Decreased in ALS (+/-)
Protein: Angiotensin precursor (SwissProt Accession number P01019)			
R.FMQAVTGWK.T (SEQ ID NO:1)	+	K.ANAGKPKDPTFIPAPIQAK.T (SEQ ID NO:43)	-
K.ALQDQLVLVAAK.L (SEQ ID NO:2)	+		
R.SLDFTELDVAAEK.I (SEQ ID NO:3)	+		
R.LQAILGVPWK.D (SEQ ID NO:4)	+		
K.QPFVQGLALYTPVVLPR.S (SEQ ID NO:5)	+		
K.VLSALQAVQGGLLVAQGR.A (SEQ ID NO:6)	+		
Protein: Kallikrein 6 (SwissProt Accession number Q92876)			
K.DSCQGDSGGPLVCGDHLR.G (SEQ ID NO:7)	+	R.LARPAKLSELIQPLPLER.D (SEQ ID NO:44)	-
K.TADGDFPDTIQCAYIHLVSR.E (SEQ ID NO:8)	+	K.LSELIQPLPLER.D (SEQ ID NO:45)	-
		R.GLVSWGNIPCGSK.E (SEQ ID NO:46)	-
		R.QRESSQEQSSVVR.A (SEQ ID NO:47)	-
		K.YGKDSCQGDSGGPLVCGDHLR.G (SEQ ID NO:48)	-
		R.AVIHPDYDAASHDQDIMLLR.L (SEQ ID NO:49)	-
Protein: Kininogen-1 (SwissProt Accession number P01042)			
R.ETTCSKESNEELTESCETK.K (SEQ ID NO:9)	+	K.RPPGFSPFR.S (SEQ ID NO:50)	-
		R.IGEIKEETTSHLR.S (SEQ ID NO:51)	-

TABLE 1-continued

Sequence	Increased/ Decreased in ALS (+/-)	Sequence	Increased/ Decreased in ALS (+/-)
		R.KLGQSLDCNAEVYVVPWEKK.I (SEQ ID NO:52)	-
		K.GRPPKAGAEPASER.E (SEQ ID NO:53)	-
		K.AATGECTATVGKR.S (SEQ ID NO:54)	-
Protein: Clusterin (activated by AT-1 receptor binding to ANG-II) (SwissProt Accession number P10909)			
R.ELDESLQVAER.L (SEQ ID NO:1)	+	R.KTLLSNLEEAK.K (SEQ ID NO:55)	-
		K.YVNKEIQNAVNGVK.Q (SEQ ID NO:56)	-
Protein: Antithrombin III (SwissProt Accession number P01008)			
K.LQPLDFKENAEQSR.A (SEQ ID NO:11)	+		
K.ATEDEGSEQKIPEATNRR.V (SEQ ID NO:12)	+		
K.SKLPGIVAEGR.D (SEQ ID NO:13)	+		
R.DDLYVSDAFHK.A (SEQ ID NO:14)	+		
K.TSDQIHFFFAK.L (SEQ ID NO:15)	+		
R.VAEGTQVLELPFK.G (SEQ ID NO:16)	+		
R.EVPLNTIIFMGR.V (SEQ ID NO:17)	+		
K.NDNDNIFLSPLSISTAFAMTK.L (SEQ ID NO:18)	+		
R.ITDVIPSEAINELTVLVLVNTIYFK.G (SEQ ID NO:19)	+		
Protein: Plasminogen (SwissProt Accession number P0074 7)			
K.RAPWCHTTNSQVR.W (SEQ ID NO:20)	+		
K.NYCRNPDGDVGGPWCYTTNPR.K (SEQ ID NO:21)	+		
Protein: Plasminogen activator, urokinase (SwissProt Accession number Q03405)			
R.GCATASMCQHAHLGDAFSMN HIDVSCCTK.S (SEQ ID NO:22)	+		
Protein: Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)			
K.TVQAVLTVPK.L (SEQ ID NO:23)	+	R.DTDTGALLFIGK.I (SEQ ID NO:57)	-
K.LQSLFDSPDFSK.I (SEQ ID NO:24)	+		
R.YGLDSDLSCK.I (SEQ ID NO:25)	+		
R.KTSLEDF YLDEER.T (SEQ ID NO:26)	+		
K.TSLEDFYLDEER.T (SEQ ID NO:27)	+		
R.ALYYDLISSPDIHGTYK.E (SEQ ID NO:28)	+		
R.LDLQEINNWVQAQMK.G (SEQ ID NO:29)	+		
K.EIPDEISILLLGVAHFK.G (SEQ ID NO:30)	+		
Protein: Vitamin D-binding protein precursor (SwissProt Accession number P02774)			
R.THLPEVKLSK.V (SEQ ID NO:31)	+	K.LPDATPTELAK.L (SEQ ID NO:58)	-
K.ELPEHTVKLCDNLSTK.N (SEQ ID NO:32)		K.ELSSFIDKGQELCADYSENTFTEYK.K (SEQ ID NO:59)	-
		K.SLGECCDVEDSTTCFNAKGPLLKK.E (SEQ ID NO:60)	-

TABLE 1-continued

Sequence	Increased/ Decreased in ALS (+/-)	Sequence	Increased/ Decreased in ALS (+/-)
Protein: Vitamin D-binding protein variant (SwissProt Accession number Q53F31)			
K.AKLPEATPTELAK.L (SEQ ID NO:33)	+		
Protein: Insulin-like growth factor binding protein 7 (IGFBP-7) (SwissProt Accession number Q16270)			
R.TELLPGDRDNLAIQTR.G (SEQ ID NO:34)	+	R.GGPEKHEVTGWVLVSPLSK.E (SEQ ID NO:61)	-
K.EDAGEYECHASNSQGQASASAK.I (SEQ ID NO:35)	+	R.GKAGAAAGGPGVSGVCVCK.S (SEQ ID NO:62)	-
		R.ITVVDALHEIPVKKGEGAEL. (SEQ ID NO:63)	-
Protein: Insulin-like growth factor binding protein 6 (IGFBP-6) (SwissProt Accession number P24592)			
R.HLDSVLQQLQTEVYR.G (SEQ ID NO:36)	+		
R.CLPARAPAVAEENPK.E (SEQ ID NO:37)	+		
R.EGQECGVYTPNCAPGLQCHPPK.D (SEQ ID NO:38)	+		
Protein: Coagulation Factor XII (SwissProt Accession number P00748)			
R.TTLSGAPCQPWASEATYR.N (SEQ ID NO:39)	+		
R.LHEAFSPVSYQHDLALLR.L (SEQ ID NO:40)	+		
R.NKPGVYTDVAYYLAWIR.E (SEQ ID NO:41)	+		
R.LCHCPVGYTGPFCDVDTK.A (SEQ ID NO:42)	+		

[0017] It will be understood that SEQ ID NOs: 1-63 represent polypeptides obtained from trypsin digestion. In performing the inventive method, alternative proteolytic enzymes can be employed (which are known to those of ordinary skill in the art), many of which cleave between amino acids differently than trypsin. Accordingly, the inventive method can employ alternative sequences comprising a majority of contiguous amino acids from any of SEQ ID NOs: 1-63, but which can also comprise additional amino acids at either or both ends. Moreover, it will be observed that a polypeptide resulting from trypsin cleavage can lack the amino and carboxy terminal amino acids of SEQ ID NOs: 1-63 (left and right of the "." in Table 1), inasmuch as these are cleaved and not present in the digested polypeptide. It should be noted, however, that SEQ ID NO:63 represents the carboxy-terminus of the mature protein, thus the polypeptide is expected to possess the terminal leucine residue.

[0018] In another aspect, the invention provides a method of determining a prognosis of a motor neuron disease by assessing a changed level of one or more proteins or polypeptides of the rennin-angiotensin pathway relative to a control. In one embodiment, the prognostic method comprises (1) providing a sample taken from a patient previously diagnosed with a motor neuron disease; (2) measuring or quantifying in the sample the amount of one or more proteins or polypeptides in the renin-angiotensin pathway, such as SEQ ID NOs: 1-42, polypeptides comprising a majority of contiguous amino acids of SEQ ID NOs: 1-42, or the proteins associated therewith (see Table 1); and (3) comparing the amount of the one or more protein or polypeptide sequences with a control. In this aspect of the invention, the presence of an increased quantity of the one or more protein or polypeptide sequences compared to the control indicates a prognosis of advancing disease, and a decreased or unchanged quantity compared to the control indicates a prognosis of remission or non-advancing disease as determined by clinical parameters.

[0019] In another embodiment, the prognostic method comprises (1) providing a sample taken from a patient previously diagnosed with a motor neuron disease; (2) measuring or quantifying in the sample the amount of one or more proteins or polypeptides in the renin-angiotensin pathway, such as SEQ ID NOs: 43-63, polypeptides comprising a majority of contiguous amino acids of SEQ ID NOs: 43-63, or proteins associated therewith; and (3) comparing the amount of the one or more protein or polypeptide sequences with a control. In this aspect of the invention, the presence of a decreased quantity of the one or more protein or polypeptide sequences compared to the control indicates a prognosis of advancing disease, and an increased or unchanged quantity compared to the control indicates a prognosis of remission or non-advancing disease as determined by clinical parameters.

[0020] In some embodiments, the prognostic method further comprises detecting aberrant post-translational modification of the one or more peptide sequences as compared to the control sample, wherein the presence of aberrant post-translational modification indicates a diagnosis of motor neuron disease. Thus, for example, the invention provides a method of determining a prognosis of a motor neuron disease

in a mammal comprising (1) providing a sample taken from a patient; (2) evaluating the sample for post-translational modification of one or more proteins or polypeptides in the renin-angiotensin pathway, (e.g., polypeptides comprising any of SEQ ID NOs: 1-63 or a majority of contiguous amino acids from SEQ ID NOs: 1-63, and the proteins associated therewith (see Table 1)); and (3) comparing the post-translational modification of the proteins or polypeptides with a control. It will be observed that, according to this aspect of the invention, increased aberrant post-translational modification of the proteins or polypeptides as compared to the control indicates a prognosis of advancing disease, and decreased level of aberrant post-translational modification of the proteins or polypeptides as compared to the control indicates a prognosis of remission or non-advancing disease as determined by clinical parameters.

[0021] The control for use in the prognostic method can be, for example, a prior sample from the patient and/or a predetermined expression profile. In other embodiments, the control is a profile of measurements understood to reflect expected levels of the peptides a subject, which can be a non-diseased subject of the same species as the patient or one with a disease at an early or advanced stage relative to the patient. In some embodiments, additional controls can be also be compared to the test sample, such as samples taken from diseased subjects or profiles of measurements understood to reflect peptide levels associated with motor neuron disease.

[0022] In any aspect of the invention, the amount of the peptide sequence(s) can be measured using any method known to one of ordinary skill in the art, such as mass spectrometry, ELISA, or Western blot. In a preferred embodiment, mass spectrometry is used. In a more preferred embodiment, the mass spectrometry is liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS). Exemplary protocols for performing LC MS/MS to quantify peptides are provided in Nagele, et al., *Exp Rev Proteomics* 1(1): 37-46 (2004); Peng J, et al., *J Proteome Res* 2(1): 43-50 (2003); and Qian W J, et al., *J Proteome Res* 4(1): 53-62 (2005). Exemplary mass spectrometry methods are also described in US Pub. No. 2005/0148026, the contents of which are incorporated herein by reference. Additional mass spectrometry based methodologies to identify peptide and protein alterations include MALDI-MS/MS (see, e.g., Pan, et al., *Anal. Chem.* 75: 1316-1324 (2003).

[0023] One of ordinary skill in the art can use any suitable statistical calculation for determining whether concentration levels of a protein or polypeptide, or levels of post-translational modification thereof, are increased or decreased relative to a control. It will be understood that levels are significantly different from control if the test sample differs from the control sample by more than 1%. In some embodiments, peptide levels may differ from the control sample by more than 5%. In other embodiments, peptide levels may differ from the control sample by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000% or more, as well as intervening values.

[0024] In performing the inventive methods, the sample can be any suitable tissue sample such as cerebrospinal fluid, blood, or urine. In some preferred embodiments, the sample is cerebrospinal fluid. The sample can be obtained by any method known to one of ordinary skill in the art.

[0025] The motor neuron disease can be any motor neuron disease, such as amyotrophic lateral sclerosis (ALS), primary lateral sclerosis, progressive muscular atrophy, pseudobulbar palsy, progressive bulbar palsy, lower motor neuron disease and spinal muscular atrophy. In a preferred embodiment, the motor neuron disease is amyotrophic lateral sclerosis (ALS).

[0026] The patient or the subject from whom a control sample is obtained can be a human or any suitable non-human mammal such as a mouse, rat, rabbit, cat, dog, pig, sheep, cow, or primate. In some embodiments, the subject is a non-human experimental animal model. In a preferred embodiment, the subject a primate. In a more preferred embodiment, the subject is a human.

[0027] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0028] This example demonstrates a method of measuring peptide biomarkers of motor neuron disease.

[0029] A sample of cerebrospinal fluid (CSF) is taken from a patient diagnosed with ALS and a control sample is taken from a non-diseased control subject. The most abundant proteins are removed by affinity chromatography and the samples digested with trypsin and peptides enriched prior to liquid chromatography mass spectrometry (LC-MS/MS).

[0030] Protein first dimension liquid chromatography (1D LC) is performed on the ALS and control samples using a 3 μL injection into a ZORBAZ 300SB-C-18 column, 5 μm particle size, 5×0.3 mm trap, or a customized gradient (Picofrit, Proteopep 2, 5 μm particle size, 75 μm ID×15 μm tip×10 cm length) for analysis on the Thermo LTQ-Orbitrap. Using these gradients, base peak chromatograms of the ALS sample and control sample are generated from a full MS scan as shown in FIG. **1** ("Comparing the Chromatograms from ALS"). Peptide identification is performed on each peak to generate a complete peptide list, and proteins determined by SEQUEST database (Human_ref.fasta, parsed from nr.fasta) comparisons. The peptide mass window is +/−2 Da, and the peptide mass range is 600-5000 Da. Monoisotopic parent and fragment ions are identified with a minimum ion count of 10. Filter applied on the search results are HUPO recommendation: XCorr>1.9, 2.2, 3.7 for 1+, 2+, 3+ peptides, respectively RSP<5, Delta CN>0.1, with greater than or equal to 2 different peptides per protein.

[0031] Protein second dimension liquid chromatography (2D LC) is performed for additional analysis of the proteome. For CSF, a 20 μL injection into a Picofrit, Thermobiobasic C18 column, 5 μm particle size, 75 μm ID×10 cm length. The column is eluted in five salt steps were used: 0 mM, 40 mM, 80 mM, 125 mM, and 800 mM. The flow rate is approximately 400 nL/min at the column tip. Each of these gradient fractions is run through the 1D column described above and peptides eluted with 2-40% acetonitrile+0.1% formic acid. Peptide identification is performed as described above [0028]. Exemplary base peak chromatograms of the ALS sample and control sample are generated as shown in FIGS. **2** and **3**, respectively ("2D LC on ALS Samples" and "2D LC on NC Samples").

[0032] Results: both 1D LC and 2D LC MS/MS indicate that the ALS sample has a noticeably different MS profile from that of the control. SIEVE software was used to analyze and identify corresponding peaks between the two samples and to identify the detected peptide sequences. In the ALS sample, one or more peptides having SEQ ID NOs: 1-42 is

detected at greater levels than in the control sample, and/or one or more peptides having SEQ ID NOs: 43-63 is detected at lower levels than in the control sample.

[0033] These results demonstrate that use of LC MS/MS can be used to determine levels of biomarker peptides of motor neuron disease.

EXAMPLE 2

[0034] This example demonstrates a method of identifying post-translational modification of peptide biomarkers of motor neuron disease.

[0035] Samples are prepared as described in Example 1 above and run in 1D and 2D LC MS/MS as described above. Post translational modification of the detected peptides is indicated through LC-MS/MS analysis using the Thermo LTQ-Orbitrap XL equipped with Electron Transfer Dissociation (ETD) to detect post-translational modifications to each identified peptide within the sample. Other methods to detect peptide post-translational modifications including purification of phosphopeptide by column chromatography and subsequent antibody detection analysis could also be utilized. In evaluating the test sample, one or more of peptides having SEQ ID NOs: 1-63 is found to have post-translational modification differing from those of the control sample.

EXAMPLE 3

[0036] This example demonstrates a method of diagnosing motor neuron disease.

[0037] A sample of CSF is taken from a patient suspected of having ALS. The sample is evaluated using 1D and 2D LC MS/MS as described in Examples 1 and 2, and compared to a control sample from a non-diseased subject. Based on the resulting base peak chromatograms, the sample is found to be elevated in one or more peptides having SEQ ID NOs: 1-42 and/or deficient in one or more peptides having SEQ ID NOs:43-63. Additionally, one or more of the peptides of SEQ ID NOs: 1-63 is found to have post-translational modification differing from those of the control sample. This supports a diagnosis of ALS.

EXAMPLE 4

[0038] This example demonstrates a method of determining a prognosis of motor neuron disease.

[0039] Two samples of CSF are taken from a patient clinically diagnosed with ALS at a six-month interval. The samples are evaluated using 1D and 2D LC MS/MS as described in Examples 1 and 2. The resulting base peak chromatograms are compared to each other, and the second sample is found to have increased amounts of one or more peptides having SEQ ID NOs: 1-42, and/or decreased amounts of one or more peptides having SEQ ID NOs:43-63. Additionally, one or more of the peptides of SEQ ID NOs: 1-63 is found to have increased post-translational modification in the second sample as opposed to the earlier, first sample. Accordingly, a prognosis of advancing ALS is made.

EXAMPLE 5

[0040] This example demonstrates a method of measuring peptide biomarkers for ALS.

[0041] A sample of cerebrospinal fluid (CSF) is taken from a patient recently diagnosed with ALS and a control subject. The control subject groups are healthy, non-diseased controls, subjects with multiple sclerosis, subjects with Alzheimer's disease, subjects with upper motor neuron disease, and subjects with lower motor neuron disease. Each control subject group is analyzed independent of the other control subject groups. The most abundant proteins are removed by affinity chromatography and the samples digested with trypsin and peptides enriched prior to liquid chromatography mass spectrometry (LC-MS/MS). Samples are analyzed on the LC-MS/MS as described in Example 1 for a 1D LC analysis.

[0042] Data (Table 2) indicates that ALS samples have specific peptide profile and levels that differentiate ALS from control subject groups. A univariate significance of peptides for each protein between all sample groups is tested by non-parametric Kruskal-Wallis test.

TABLE 2

PROTEIN	SwissProt #	SEQ ID NO:	p value
Clusterin	P10909	10, 55, 56	0.0201
Antithrombin III variant	Q7KZ97	11-19	0.0312
Plasminogen	P00747	20, 21	0.0106
Coagulation Factor XII	P00748	39-42	0.0158

[0043] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0044] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0045] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

SEQUENCE LISTING

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<222> LOCATION: (1)..(11)
<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor
(SwissProt Accession number P01019)

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Arg Phe Met Gln Ala Val Thr Gly Trp Lys Thr
1 5 10

<210> SEQ ID NO 2
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor
(SwissProt Accession number P01019)

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<210> SEQ ID NO 3
<211> LENGTH: 15
<212> TYPE: PRT
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<220> FEATURE:
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<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor
(SwissProt Accession number P01019)

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Arg Ser Leu Asp Phe Thr Glu Leu Asp Val Ala Ala Glu Lys Ile
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<210> SEQ ID NO 4
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<222> LOCATION: (1)..(12)
<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor
(SwissProt Accession number P01019)

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Arg Leu Gln Ala Ile Leu Gly Val Pro Trp Lys Asp
1 5 10

<210> SEQ ID NO 5
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<220> FEATURE:
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<222> LOCATION: (1)..(19)

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<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor (SwissProt Accession number P01019)

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Lys Gln Pro Phe Val Gln Gly Leu Ala Leu Tyr Thr Pro Val Val Leu
1 5 10 15

Pro Arg Ser

<210> SEQ ID NO 6
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor (SwissProt Accession number P01019)

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1 5 10 15

Gln Gly Arg Ala
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<210> SEQ ID NO 7
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6 (SwissProt Accession number Q92876)

<400> SEQUENCE: 7

Lys Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Gly Asp
1 5 10 15

His Leu Arg Gly
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<210> SEQ ID NO 8
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6 (SwissProt Accession number Q92876)

<400> SEQUENCE: 8

Lys Thr Ala Asp Gly Asp Phe Pro Asp Thr Ile Gln Cys Ala Tyr Ile
1 5 10 15

His Leu Val Ser Arg Glu
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<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: Polypeptide from Kininogen-1 (SwissProt Accession number P01042)

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Arg Glu Thr Thr Cys Ser Lys Glu Ser Asn Glu Glu Leu Thr Glu Ser
1 5 10 15

Cys Glu Thr Lys Lys
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<210> SEQ ID NO 10
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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(13)
<223> OTHER INFORMATION: Polypeptide from Clusterin (activated by AT-1 receptor binding to ANG-II) (SwissProt Accession number P10909)

<400> SEQUENCE: 10

Arg Glu Leu Asp Glu Ser Leu Gln Val Ala Glu Arg Leu
1 5 10

<210> SEQ ID NO 11
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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(16)
<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 11

Lys Leu Gln Pro Leu Asp Phe Lys Glu Asn Ala Glu Gln Ser Arg Ala
1 5 10 15

<210> SEQ ID NO 12
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<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

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Lys Ala Thr Glu Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr
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Asn Arg Arg Val
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<210> SEQ ID NO 13
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<212> TYPE: PRT
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<220> FEATURE:
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<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 13

Lys Ser Lys Leu Pro Gly Ile Val Ala Glu Gly Arg Asp
1 5 10

<210> SEQ ID NO 14
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 14

Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe His Lys Ala
1 5 10

<210> SEQ ID NO 15
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(13)
<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 15

Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu
1 5 10

<210> SEQ ID NO 16
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 16

Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu Pro Phe Lys Gly
1 5 10 15

<210> SEQ ID NO 17
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

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<400> SEQUENCE: 17

Arg Glu Val Pro Leu Asn Thr Ile Ile Phe Met Gly Arg Val
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<210> SEQ ID NO 18
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 18

Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro Leu Ser Ile Ser Thr
1 5 10 15

Ala Phe Ala Met Thr Lys Leu
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<210> SEQ ID NO 19
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(27)
<223> OTHER INFORMATION: Polypeptide from Antithrombin III (SwissProt Accession number P01008)

<400> SEQUENCE: 19

Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn Glu Leu Thr Val
1 5 10 15

Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly
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<210> SEQ ID NO 20
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Plasminogen (SwissProt Accession number P00747)

<400> SEQUENCE: 20

Lys Arg Ala Pro Trp Cys His Thr Thr Asn Ser Gln Val Arg Trp
1 5 10 15

<210> SEQ ID NO 21
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: Polypeptide from Plasminogen (SwissProt Accession number P00747)

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<400> SEQUENCE: 21

Lys Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys
1 5 10 15

Tyr Thr Thr Asn Pro Arg Lys
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<210> SEQ ID NO 22
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(31)
<223> OTHER INFORMATION: Polypeptide from Plasminogen activator, urokinase (SwissProt Accession number Q03405)

<400> SEQUENCE: 22

Arg Gly Cys Ala Thr Ala Ser Met Cys Gln His Ala His Leu Gly Asp
1 5 10 15

Ala Phe Ser Met Asn His Ile Asp Val Ser Cys Cys Thr Lys Ser
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(12)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 23

Lys Thr Val Gln Ala Val Leu Thr Val Pro Lys Leu
1 5 10

<210> SEQ ID NO 24
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 24

Lys Leu Gln Ser Leu Phe Asp Ser Pro Asp Phe Ser Lys Ile
1 5 10

<210> SEQ ID NO 25
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(12)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

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<400> SEQUENCE: 25

Arg Tyr Gly Leu Asp Ser Asp Leu Ser Cys Lys Ile
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<210> SEQ ID NO 26
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 26

Arg Lys Thr Ser Leu Glu Asp Phe Tyr Leu Asp Glu Glu Arg Thr
1 5 10 15

<210> SEQ ID NO 27
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 27

Lys Thr Ser Leu Glu Asp Phe Tyr Leu Asp Glu Glu Arg Thr
1 5 10

<210> SEQ ID NO 28
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 28

Arg Ala Leu Tyr Tyr Asp Leu Ile Ser Ser Pro Asp Ile His Gly Thr
1 5 10 15

Tyr Lys Glu

<210> SEQ ID NO 29
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(17)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

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<400> SEQUENCE: 29

Arg Leu Asp Leu Gln Glu Ile Asn Asn Trp Val Gln Ala Gln Met Lys
1 5 10 15

Gly

<210> SEQ ID NO 30
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 30

Lys Glu Ile Pro Asp Glu Ile Ser Ile Leu Leu Leu Gly Val Ala His
1 5 10 15

Phe Lys Gly

<210> SEQ ID NO 31
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(12)
<223> OTHER INFORMATION: Polypeptide from Vitamin D-binding protein precursor (SwissProt Accession number P02774)

<400> SEQUENCE: 31

Arg Thr His Leu Pro Glu Val Lys Leu Ser Lys Val
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(18)
<223> OTHER INFORMATION: Polypeptide from Vitamin D-binding protein precursor (SwissProt Accession number P02774)

<400> SEQUENCE: 32

Lys Glu Leu Pro Glu His Thr Val Lys Leu Cys Asp Asn Leu Ser Thr
1 5 10 15

Lys Asn

<210> SEQ ID NO 33
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Vitamin D-binding protein variant (SwissProt Accession number Q53F31)

<400> SEQUENCE: 33

Lys Ala Lys Leu Pro Glu Ala Thr Pro Thr Glu Leu Ala Lys Leu
1 5 10 15

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<210> SEQ ID NO 34
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(18)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 7 (IGFBP-7) (SwissProt Accession number Q16270)

<400> SEQUENCE: 34

Arg Thr Glu Leu Leu Pro Gly Asp Arg Asp Asn Leu Ala Ile Gln Thr
1 5 10 15

Arg Gly

<210> SEQ ID NO 35
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(24)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 7 (IGFBP-7) (SwissProt Accession number Q16270)

<400> SEQUENCE: 35

Lys Glu Asp Ala Gly Glu Tyr Glu Cys His Ala Ser Asn Ser Gln Gly
1 5 10 15

Gln Ala Ser Ala Ser Ala Lys Ile
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<210> SEQ ID NO 36
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(17)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 6 (IGFBP-6) (SwissProt Accession number P24592)

<400> SEQUENCE: 36

Arg His Leu Asp Ser Val Leu Gln Gln Leu Gln Thr Glu Val Tyr Arg
1 5 10 15

Gly

<210> SEQ ID NO 37
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(17)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 6 (IGFBP-6) (SwissProt Accession number P24592)

<400> SEQUENCE: 37

Arg Cys Leu Pro Ala Arg Ala Pro Ala Val Ala Glu Glu Asn Pro Lys
1 5 10 15

Glu

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<210> SEQ ID NO 38
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(24)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 6 (IGFBP-6) (SwissProt Accession number P24592)

<400> SEQUENCE: 38

Arg Glu Gly Gln Glu Cys Gly Val Tyr Thr Pro Asn Cys Ala Pro Gly
1 5 10 15

Leu Gln Cys His Pro Pro Lys Asp
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<210> SEQ ID NO 39
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Polypeptide from Coagulation Factor XII (SwissProt Accession number P00748)

<400> SEQUENCE: 39

Arg Thr Thr Leu Ser Gly Ala Pro Cys Gln Pro Trp Ala Ser Glu Ala
1 5 10 15

Thr Tyr Arg Asn
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<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Polypeptide from Coagulation Factor XII (SwissProt Accession number P00748)

<400> SEQUENCE: 40

Arg Leu His Glu Ala Phe Ser Pro Val Ser Tyr Gln His Asp Leu Ala
1 5 10 15

Leu Leu Arg Leu
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<210> SEQ ID NO 41
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<220> FEATURE:
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<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION: Polypeptide from Coagulation Factor XII (SwissProt Accession number P00748)

<400> SEQUENCE: 41

Arg Asn Lys Pro Gly Val Tyr Thr Asp Val Ala Tyr Tyr Leu Ala Trp
1 5 10 15

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<210> SEQ ID NO 42
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<212> TYPE: PRT
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Polypeptide from Coagulation Factor XII
(SwissProt Accession number P00748)

<400> SEQUENCE: 42

Arg Leu Cys His Cys Pro Val Gly Tyr Thr Gly Pro Phe Cys Asp Val
1 5 10 15

Asp Thr Lys Ala
20

<210> SEQ ID NO 43
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: Polypeptide from Angiotensin precursor
(SwissProt Accession number P01019)

<400> SEQUENCE: 43

Lys Ala Asn Ala Gly Lys Pro Lys Asp Pro Thr Phe Ile Pro Ala Pro
1 5 10 15

Ile Gln Ala Lys Thr
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<210> SEQ ID NO 44
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6
(SwissProt Accession number Q92876)

<400> SEQUENCE: 44

Arg Leu Ala Arg Pro Ala Lys Leu Ser Glu Leu Ile Gln Pro Leu Pro
1 5 10 15

Leu Glu Arg Asp
20

<210> SEQ ID NO 45
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6
(SwissProt Accession number Q92876)

<400> SEQUENCE: 45

Lys Leu Ser Glu Leu Ile Gln Pro Leu Pro Leu Glu Arg Asp
1 5 10

<210> SEQ ID NO 46
<211> LENGTH: 15
<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6
(SwissProt Accession number Q92876)

<400> SEQUENCE: 46

Arg Gly Leu Val Ser Trp Gly Asn Ile Pro Cys Gly Ser Lys Glu
1 5 10 15

<210> SEQ ID NO 47
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6
(SwissProt Accession number Q92876)

<400> SEQUENCE: 47

Arg Gln Arg Glu Ser Ser Gln Glu Gln Ser Ser Val Val Arg Ala
1 5 10 15

<210> SEQ ID NO 48
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6
(SwissProt Accession number Q92876)

<400> SEQUENCE: 48

Lys Tyr Gly Lys Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val
1 5 10 15

Cys Gly Asp His Leu Arg Gly
20

<210> SEQ ID NO 49
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Polypeptide from Kallikrein 6
(SwissProt Accession number Q92876)

<400> SEQUENCE: 49

Arg Ala Val Ile His Pro Asp Tyr Asp Ala Ala Ser His Asp Gln Asp
1 5 10 15

Ile Met Leu Leu Arg Leu
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<210> SEQ ID NO 50
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(11)
<223> OTHER INFORMATION: Polypeptide from Kininogen-1
(SwissProt Accession number P01042)

-continued

<400> SEQUENCE: 50

Lys Arg Pro Pro Gly Phe Ser Pro Phe Arg Ser
1 5 10

<210> SEQ ID NO 51
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Kininogen-1
(SwissProt Accession number P01042)

<400> SEQUENCE: 51

Arg Ile Gly Glu Ile Lys Glu Glu Thr Thr Ser His Leu Arg Ser
1 5 10 15

<210> SEQ ID NO 52
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Polypeptide from Kininogen-1
(SwissProt Accession number P01042)

<400> SEQUENCE: 52

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

Pro Trp Glu Lys Lys Ile
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<210> SEQ ID NO 53
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(16)
<223> OTHER INFORMATION: Polypeptide from Kininogen-1
(SwissProt Accession number P01042)

<400> SEQUENCE: 53

Lys Gly Arg Pro Pro Lys Ala Gly Ala Glu Pro Ala Ser Glu Arg Glu
1 5 10 15

<210> SEQ ID NO 54
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: Polypeptide from Kininogen-1
(SwissProt Accession number P01042)

<400> SEQUENCE: 54

Lys Ala Ala Thr Gly Glu Cys Thr Ala Thr Val Gly Lys Arg Ser
1 5 10 15

-continued

<210> SEQ ID NO 55
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(13)
<223> OTHER INFORMATION: Polypeptide from Clusterin (activated by AT-1 receptor binding to ANG-II) (SwissProt Accession number P10909)

<400> SEQUENCE: 55

Arg Lys Thr Leu Leu Ser Asn Leu Glu Glu Ala Lys Lys
1 5 10

<210> SEQ ID NO 56
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(16)
<223> OTHER INFORMATION: Polypeptide from Clusterin (activated by AT-1 receptor binding to ANG-II) (SwissProt Accession number P10909)

<400> SEQUENCE: 56

Lys Tyr Val Asn Lys Glu Ile Gln Asn Ala Val Asn Gly Val Lys Gln
1 5 10 15

<210> SEQ ID NO 57
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: Polypeptide from Pigment epithelial derived factor precursor (PEDF or Serpin F1) (SwissProt Accession number P36955)

<400> SEQUENCE: 57

Arg Asp Thr Asp Thr Gly Ala Leu Leu Phe Ile Gly Lys Ile
1 5 10

<210> SEQ ID NO 58
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(13)
<223> OTHER INFORMATION: Polypeptide from Vitamin D-binding protein precursor (SwissProt Accession number P02774)

<400> SEQUENCE: 58

Lys Leu Pro Asp Ala Thr Pro Thr Glu Leu Ala Lys Leu
1 5 10

<210> SEQ ID NO 59
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(27)
<223> OTHER INFORMATION: Polypeptide from Vitamin D-binding protein precursor (SwissProt Accession number P02774)

-continued

<400> SEQUENCE: 59

Lys Glu Leu Ser Ser Phe Ile Asp Lys Gly Gln Glu Leu Cys Ala Asp
1 5 10 15

Tyr Ser Glu Asn Thr Phe Thr Glu Tyr Lys Lys
20 25

<210> SEQ ID NO 60
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(26)
<223> OTHER INFORMATION: Polypeptide from Vitamin D-binding protein precursor (SwissProt Accession number P02774)

<400> SEQUENCE: 60

Lys Ser Leu Gly Glu Cys Cys Asp Val Glu Asp Ser Thr Thr Cys Phe
1 5 10 15

Asn Ala Lys Gly Pro Leu Leu Lys Lys Glu
20 25

<210> SEQ ID NO 61
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 7 (IGFBP-7) (SwissProt Accession number Q16270)

<400> SEQUENCE: 61

Arg Gly Gly Pro Glu Lys His Glu Val Thr Gly Trp Val Leu Val Ser
1 5 10 15

Pro Leu Ser Lys Glu
20

<210> SEQ ID NO 62
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 7 (IGFBP-7) (SwissProt Accession number Q16270)

<400> SEQUENCE: 62

Arg Gly Lys Ala Gly Ala Ala Ala Gly Gly Pro Gly Val Ser Gly Val
1 5 10 15

Cys Val Cys Lys Ser
20

<210> SEQ ID NO 63
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: Polypeptide from Insulin-like growth factor binding protein 7 (IGFBP-7) (SwissProt Accession number Q16270)

-continued

<400> SEQUENCE: 63

Arg Ile Thr Val Val Asp Ala Leu His Glu Ile Pro Val Lys Lys Gly
1 5 10 15

Glu Gly Ala Glu Leu
20

1. A method of determining a diagnosis of motor neuron disease comprising:

providing a sample taken from a patient;

quantifying in the sample the amount of one or more proteins or polypeptides in the renin-angiotensin pathway; and

comparing the amount of the one or more proteins or polypeptides with a negative control;

wherein a different quantity of the one or more proteins or polypeptides compared to the negative control indicates a diagnosis of motor neuron disease.

2. The method of claim 1, wherein the one or more proteins or polypeptides comprises a majority of contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-42; and wherein the presence of an increased quantity of the one or more proteins or polypeptides compared to the negative control indicates a diagnosis of motor neuron disease.

3. The method of claim 1, wherein the one or more proteins or polypeptides comprises a majority of contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs:43-63; and wherein the presence of a decreased quantity of the one or more proteins or polypeptides compared to the negative control indicates a diagnosis of motor neuron disease.

4. The method of claim 1, further comprising detecting aberrant post-translational modification of the one or more proteins or polypeptides, wherein the presence of aberrant post-translational modification indicates a diagnosis of motor neuron disease.

5. The method of claim 1, wherein the sample is a sample selected from the group consisting of cerebrospinal fluid, blood, or urine.

6. The method of claim 1, wherein the motor neuron disease is selected from the group consisting of amyotrophic lateral sclerosis (ALS), primary lateral sclerosis, progressive muscular atrophy, pseudobulbar palsy, progressive bulbar palsy, lower motor neuron disease and spinal muscular atrophy.

7. The method of claim 1, wherein the negative control is a sample taken from a non-diseased subject of the same species as the patient.

8. The method of claim 1, wherein the patient is a human.

9. The method of claim 1, wherein the one or more proteins or polypeptides comprises a protein selected from the group consisting of angiotensin precursor, kallikrein 6 (isoform B and isoform A preproprotein), kininogen, clusterin, antihrombin III (Serpin C1), plasminogen, plasminogen activator/urokinase, pigment epithelial derived factor precursor (PEDF or Serpin F1), vitamin D binding protein precursor, Vitamin D binding protein variant, angiotensin preproprotein, insulin-like growth factor binding protein 6, insulin-like growth factor binding protein 7, coagulation factor II precursor, coagulation factor XII, and plasminogen activator (urokinase receptor isoform).

10. A method of diagnosing motor neuron disease comprising:

providing a sample taken from a patient;

evaluating the sample for post-translational modification of one or more proteins or polypeptides in the renin-angiotensin pathway; and

comparing the amount of aberrant post-translational modification of the one or more proteins or polypeptides with a negative control;

wherein aberrant post-translational modification of the peptides as compared to a negative control indicates a diagnosis of motor neuron disease.

11. The method of claim 10, wherein the sample is a sample selected from the group consisting of cerebrospinal fluid, blood, or urine.

12. The method of claim 10, wherein the motor neuron disease is selected from the group consisting of ALS, primary lateral sclerosis, progressive muscular atrophy, pseudobulbar palsy, progressive bulbar palsy, lower motor neuron disease and spinal muscular atrophy.

13. The method of claim 10, wherein the negative control is a sample taken from a non-diseased subject of the same species as the patient.

14. The method of claim 10, wherein the patient is a human.

15. The method of claim 10, wherein the one or more proteins or polypeptides comprises a protein selected from the group consisting of angiotensin precursor, kallikrein 6 (isoform B and isoform A preproprotein), kininogen, clusterin, antihrombin III (Serpin C1), plasminogen, plasminogen activator/urokinase, pigment epithelial derived factor precursor (PEDF or Serpin F1), vitamin D binding protein precursor, Vitamin D binding protein variant, angiotensin preproprotein, insulin-like growth factor binding protein 6, insulin-like growth factor binding protein 7, coagulation factor II precursor, coagulation factor XII, and plasminogen activator (urokinase receptor isoform).

16. The method of claim 10, wherein the one or more proteins or polypeptides comprises a majority of contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-63.

17. A method of determining a prognosis of a motor neuron disease comprising:

providing a sample taken from a patient previously diagnosed with a motor neuron disease;

quantifying in the sample the amount of one or more proteins or polypeptides in the renin-angiotensin pathway; and

comparing the amount of the one or more proteins or polypeptides with a control selected from the group

consisting of a prior sample from the same patient and a pre-determined expression profile;

wherein a different quantity of the one or more proteins or polypeptides compared to the control indicates a prognosis of advancing disease as determined by clinical parameters.

18. The method of claim **17**, wherein the one or more proteins or polypeptides comprises a majority of contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-42 and wherein the presence of an increased quantity of the one or more protein or polypeptide sequences compared to the control indicates a prognosis of advancing disease, and a decreased or unchanged quantity compared to the control indicates a prognosis of remission or non-advancing disease as determined by clinical parameters.

19. The method of claim **17**, wherein the one or more proteins or polypeptides comprises a majority of contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs:43-63 and wherein the presence of a decreased quantity of the one or more protein or polypeptide sequences compared to the control indicates a prognosis of advancing disease, and an increased or unchanged quantity compared to the control indicates a prognosis of remission or non-advancing disease as determined by clinical parameters.

20. The method of claim **17**, further comprising detecting aberrant post-translational modification of the one or more proteins or polypeptides, wherein the presence of aberrant post-translational modification indicates a prognosis of advancing motor neuron disease.

21. The method of claim **17**, wherein the sample is a sample selected from the group consisting of cerebrospinal fluid, blood, or urine.

22. The method of claim **17**, wherein the motor neuron disease is selected from the group consisting of ALS, primary lateral sclerosis, progressive muscular atrophy, pseudobulbar palsy, progressive bulbar palsy, lower motor neuron disease and spinal muscular atrophy.

23. The method of claim **17**, wherein the negative control is a sample taken from a non-diseased subject of the same species as the patient.

24. The method of claim **17**, wherein the patient is a human.

25. The method of claim **17**, wherein the one or more proteins or polypeptides comprises a protein selected from the group consisting of angiotensin precursor, kallikrein 6 (isoform B and isoform A preproprotein), kininogen, clusterin, antihrombin III (Serpin C1), plasminogen, plasminogen activator/urokinase, pigment epithelial derived factor precursor (PEDF or Serpin F1), vitamin D binding protein precursor, Vitamin D binding protein variant, angiotensin preproprotein, insulin-like growth factor binding protein 6, insulin-like growth factor binding protein 7, coagulation factor II precursor, coagulation factor XII, and plasminogen activator (urokinase receptor isoform).

26. A method of determining a prognosis of a motor neuron disease comprising:

providing a sample taken from a patient;

evaluating the sample for post-translational modification of one or more proteins or polypeptides in the renin-angiotensin pathway; and

comparing the amount of aberrant post-translational modification of the one or more proteins or polypeptides with a control selected from the group consisting of a prior sample from the same subject and a pre-determined expression profile;

wherein increased aberrant post-translational modification of the one or more proteins or polypeptides compared to the control indicates a prognosis of advancing disease, and an increased or unchanged quantity compared to the control indicates a prognosis of remission or non-advancing disease as determined by clinical parameters.

27. The method of claim **26**, wherein the sample is a sample selected from the group consisting of cerebrospinal fluid, blood, or urine.

28. The method of claim **26**, wherein the motor neuron disease is selected from the group consisting of ALS, primary lateral sclerosis, progressive muscular atrophy, pseudobulbar palsy, progressive bulbar palsy, lower motor neuron disease and spinal muscular atrophy.

29. The method of claim **26**, wherein the negative control is a sample taken from a non-diseased subject of the same species as the patient.

30. The method of claim **26**, wherein the patient is a human.

31. The method of claim **26**, wherein the one or more proteins or polypeptides comprises a protein selected from the group consisting of angiotensin precursor, kallikrein 6 (isoform B and isoform A preproprotein), kininogen, clusterin, antihrombin III (Serpin C1), plasminogen, plasminogen activator/urokinase, pigment epithelial derived factor precursor (PEDF or Serpin F1), vitamin D binding protein precursor, Vitamin D binding protein variant, angiotensin preproprotein, insulin-like growth factor binding protein 6, insulin-like growth factor binding protein 7, coagulation factor II precursor, coagulation factor XII, and plasminogen activator (urokinase receptor isoform).

32. The method of claim **26**, wherein the one or more proteins or polypeptides comprises a majority of contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-63.

* * * * *

专利名称(译)	运动神经元疾病的生物标志物		
公开(公告)号	US20090104639A1	公开(公告)日	2009-04-23
申请号	US12/209899	申请日	2008-09-12
[标]申请(专利权)人(译)	匹兹堡大学		
申请(专利权)人(译)	匹兹堡大学 - 英联邦系统的高等教育的		
当前申请(专利权)人(译)	匹兹堡大学 - 英联邦系统的高等教育的		
[标]发明人	BOWSER ROBERT P		
发明人	BOWSER, ROBERT P.		
IPC分类号	C12Q1/37 G01N33/53 C12Q1/02		
CPC分类号	G01N2800/28 G01N33/6896		
优先权	60/971709 2007-09-12 US		
外部链接	Espacenet USPTO		

摘要(译)

本发明提供了确定哺乳动物运动神经元疾病的诊断或预后的方法，包括测定取自受试者的样品中一种或多种肾素 - 血管紧张素系统的蛋白质或多肽的表达水平。类似地，与阴性对照相比，蛋白质或多肽的异常翻译后修饰表明疾病的诊断。