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(54) **DIAGNOSIS AND MONITORING OF DISEASES**
DIAGNOSE UND ÜBERWACHUNG VON KRANKHEITEN
DIAGNOSTIC ET CONTROLE DE MALADIES

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EP 1 571 970 B1

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- **PRAKASH ET AL.: ’Synthesis and Biological Activity of Novel Neuroprotective Diketopiperazines’ BIOORGANIC & MEDICINAL CHEMISTRY vol. 10, no. 9, September 2002, pages 3043 - 3048, XP002311146**
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Description

FIELD OF THE INVENTION

[0001] The invention relates to the diagnosis and monitoring of Multiple Sclerosis (MS) by quantitating Asp-Ala DKP (DA-DKP), N-acetyl-Ala-Ser DKP (NAS-DKP), N-acetyl-Ala-phophorylated-Ser DKP, Arg-Arg DKP, Gln-Asn DKP. In particular, the invention relates to the detection and measurement of the diketopiperazines Asp-Ala DKP (DA-DKP), N-acetyl-Ala-Ser DKP (NAS-DKP), N-acetyl-Ala-phophorylated-Ser DKP, Arg-Arg DKP, Gln-Asn DKP.

BACKGROUND

[0002] Simpler and faster tests for diagnosing and monitoring diseases and medical conditions are always needed. In addition, many serious illnesses remain difficult to diagnose and monitor, and methods of diagnosing and monitoring these diseases and conditions are critically needed.

[0003] For instance, multiple sclerosis (MS) is difficult to diagnose because the progress, severity and specific symptoms of MS are quite variable and unpredictable. There are not laboratory tests, symptoms or physical findings that can, by themselves, determine if a person has MS.

[0004] The long established criteria for diagnosing MS are:

1. There must be objective evidence of two attacks (*i.e.*, two episodes of demyelination in the central nervous system (CNS)). An attack (also known as an exacerbation, flare or relapse) is defined clinically as the sudden appearance or worsening of an MS symptom or symptoms, which lasts at least 24 hours. The objective evidence comes from findings of a neurological examination and additional tests.
2. The two attacks must be separated in time (at least one month apart) and space (indicated by evidence of inflammation and/or damage in different areas of the-CNS).
3. There must be no other explanation for these attacks or the symptoms the person is experiencing. Many symptoms that are common in MS can also be caused by other diseases. Therefore, the MS diagnosis can only be made by carefully ruling out all possibilities.

[0005] Over the last twenty years, tests such as magnetic resonance imaging (MRI), examination of cerebrospinal fluid, and evoked response testing have played an increasingly important role in the diagnostic process. In 2001, the International Panel on the Diagnosis of Multiple Sclerosis issued a revised set of diagnostic criteria (Annals of Neurology, 50:121-127 (2001)). In addition to the traditional requirements given above, the revised criteria provide specific guidelines for using findings of MRI, cerebrospinal fluid analysis and visual evoked potentials to provide evidence of the second attack and thereby confirm the diagnosis more quickly. These guidelines also facilitate the diagnostic process in those patients who have had steady progression of disability without distinct attacks. However, even with these revised criteria, diagnosis of MS is still difficult and still typically takes several months or even years.

[0006] Due to the possibility of worsening or recurrence of MS, making a conclusive diagnosis quickly would be of great benefit. Drugs for the treatment of MS are now available which slow or prevent progression of the disease in many patients, and a quick diagnosis would allow early intervention and could significantly improve the prognosis for many MS patients.

[0007] The diagnosis of Alzheimer's disease is difficult and often relies on the exclusion of other causes. Various cognitive tests are employed to possibly identify the disease. However, a definitive diagnosis is only possible by a brain autopsy after death. Clearly, a diagnostic test that can provide a diagnosis for living Alzheimer's disease patients is is needed.

[0008] Brain ischemia is currently a clinical diagnosis. Although certain biochemical markers have been described, such as Enolase, S-100 family of proteins and others, the imaging techniques available to the clinician are more reliable and specific. A reliable and specific biochemical marker for brain ischemia would be helpful in the diagnosis and monitoring of this disease.

[0009] Early cardiac ischemia is also difficult to diagnose. Cardiac markers of cellular necrosis, such as creatine kinase isoenzymes (CK-MB), myoglobin, or troponin, are unreliable markers of transient myocardial ischemia, particularly when measured in the first 2 to 6 hours after an ischemic event. Kontos, M.C. and R.L. Jesse, Am J Cardiol, 2000. 85(5A): p. 32B-39B; Ishikawa, Y., et al., Clin Chem, 1997. 43(3): p. 467-75; Brogan, G.X., Jr., et al., Acad Emerg Med, 1997. 4(1): p. 6-12; Hedges, J.R., et al., Acad Emerg Med, 1996. 3(1): p. 27-33. Patients who are examined soon after the **onset** of ischemic symptoms typically require prolonged observation to rule out myocardial infarction or myocardial ischemia. Gomez, M.A., et al., J Am Coll Cardiol, 1996. 28(1): p. 25-33; Zalenski, R.J., et al., Arch Intern Med, 1997. 157(10): p. 1085-91; de Winter, R.J., et al., Ann Emerg Med, 2000. 35(2): p. 113-20; Peacock, W.I., et al. Ann Emerg Med, 2000. 35(3): p. 213-20.

[0010] A novel blood assay method to measure reduced exogenous cobalt binding to human serum albumin in patients with myocardial ischemia has been described. Bar-Or et al., J. Emerg. Med., 2000. 19(4): p. 311-5. The albumin-cobalt binding (ACB) assay measures the binding capacity of exogenous cobalt to the amino terminus (N-terminus) of human albumin. Under normal conditions, transition metals, including cobalt, are tightly bound to the exposed N-terminus of albumin. Kubal, G., P.J. Sadler, and A. Tucker, Eur J Biochem, 1994. 220(3): p. 781-7. The ACB assay is based on observations that ischemic conditions may alter the N-terminus of albumin and rapidly reduce its binding capacity for transition metals. Berenshtein et al., J. Mol. Cell. Cardiol., 1997. 29(11): p. 3025-34; Bar-Or et al., Eur. J. Biochem., 2001. 268(1): p. 42-47. Ischemia-induced alterations to albumin would be predicted to occur minutes or hours before abnormal levels of CK-MB, myoglobin, or troponin could be detected. However, the ACB assay has been approved only as a test to rule out cardiac ischemia, and it would be highly desirable to have an assay that could diagnose cardiac ischemia, as well as rule it out.

[0011] Low birth weight (LBW) is the leading cause of fetal and neonatal morbidity and mortality worldwide. LBW is generally accepted to indicate a weight of less than 2500 grams at delivery, and may result from a newborn being born at term but small for gestational age (SGA), being born preterm and appropriate for gestational age (AGA) or being both preterm and SGA. As such, the epidemiology of LBW is complex and multifactorial.

[0012] SGA is a statistical definition, indicating that the birth weight is less than the tenth percentile for gestational age. By definition then, 10% of newborns are SGA. In practice, some of these newborns are small and well, fulfilling their genetic growth potential, and are not at substantial risk. Other SGA newborns on the other hand are truly growth impaired, failing to meet their genetic growth potential due to a variety of factors as discussed below. These newborns are said to suffer from fetal growth restriction (FGR). In practice, some infants are presumably AGA and suffer from FGR; that is to say their weight may be at the 20th percentile for gestational age, but they were genetically programmed to weigh at the 80th percentile. These infants are difficult to identify in a practical sense, as there is no a priori way of knowing how much an individual "should" weigh.

[0013] FGR leads to LBW both by direct impairment of fetal growth, and often in addition by necessitating indicated preterm delivery due to compromised fetal status or associated maternal disease (*e.g.*, preeclampsia). Morbidity due to LBW and/or prematurity is varied and substantial and well documented elsewhere. Additionally, recent data have suggested that a compromised intrauterine environment can have a profound influence on health in adult life, the so-called "fetal origins of disease" or Barker hypothesis. Via these various mechanisms, the disease burden attributable to FGR is tremendous.

[0014] While the fetus/neonate is often the focus of concern in pregnancies complicated by FGR, it is important to recall that these pregnancies are also often complicated by conditions that directly threaten maternal health. Most notably, preeclampsia, whose precise pathophysiology remains obscure, has long been felt to result from placental ischemia. Preeclampsia and its complications are the leading causes of maternal mortality worldwide.

[0015] While the differential diagnosis of FGR is diverse, including chromosomal, toxic, viral and other etiologies, the majority of cases result from uteroplacental insufficiency (UPI). UPI may be associated with a variety of maternal diseases (hypertension, renal disease, systemic lupus erythematosus, antiphospholipid syndrome, thrombophilia, etc.), pregnancy complications (placental abruption, preeclampsia), or may be idiopathic. Regardless of the etiology, the presumed unifying underlying pathophysiology results from reduced placental blood flow (ischemia) in either the maternal or the fetal circulation, or both.

[0016] As a crude measure, it is known that there is a direct relationship between placental weight and fetal weight, suggesting that placental resources might control fetal growth to some extent. There are a large number of placental pathologic lesions associated with FGR. In general, these are lesions that would be expected to compromise maternal and/or fetal blood flow. The association between reduced maternal and/or fetal blood flow (ischemia) and FGR is also corroborated by a large amount of Doppler flow data in affected pregnancies. In many cases, these abnormal Doppler flow waveforms correlate well with abnormal placental pathology.

[0017] While much is known about the pathophysiology of FGR, much remains to be understood. In the clinical setting, although various risk factors for FGR are recognized, their positive predictive values and sensitivities are limited. There can be difficulty differentiating the FGR fetus from the "SGA but well" fetus. Recognizing this difference is important to avoid unnecessary interventions on well pregnancies. Early identification of pregnancies destined to be affected by FGR might help foster appropriate follow-up. Timing of delivery is also a matter of intense interest, balancing the benefits of advancing gestation against those of continuing in an ischemic environment. Finally, on a more fundamental level, access to a clinical test to identify placental ischemia and quantify its severity might ultimately help foster appropriate treatment or even prevention.

[0018] As noted above, the ACB assay for ruling out ischemia is based on observations that ischemic conditions may alter the N-terminus of human serum albumin and rapidly reduce its binding capacity for transition metals. The nature of the alterations of the N-terminus of human serum albumin that may account for its reduced metal binding capacity have not been identified, but cleavage of 1-4 amino acids has been proposed as one of several possibilities. See PCT application WO 00/20840. In particular, it has been hypothesized that cleavage of the N-terminal dipeptide (Asp-Ala or

DA) from human serum albumin and the cyclization of the dipeptide to form the diketopiperazine (DA-DKP) may partially explain the observation of reduced metal binding to N-terminus of human serum albumin in ischemia. Bar-Or et al., Biochem. Biophys. Res. Commun., 84:856-862 (June 15, 2001). However, this article does not teach or suggest that DA-DKP can be used as a marker of ischemia.

[0019] PCT application WO 00/20454 discloses a marker for free radical damage. The marker is human serum albumin whose N-terminal metal binding site has been modified by free radical damage. Reduced metal binding to the altered N-terminus is used to detect and measure the free radical damage. Several possible modifications of the N-terminus of human serum albumin that might account for the reduced metal binding are proposed, including the possibility that the N-terminal dipeptide (DA) is cleaved by free radicals and that this dipeptide then cyclizes to form DA-DKP. Although direct detection of the altered N-terminus of human serum albumin is suggested as a method of detecting and measuring free radical damage, measurement of the hypothetical DA-DKP is not taught or suggested for this purpose.

[0020] HisPro-DKP was a known diagnostic marker for (Jackson et al, 1986. Neurol, 36, 1218-1223). Cyclo(His-Pro) was used as marker for schizophrenia (Prasad et al, 1991. Neuropeptides, 20, 187-190). WO02062797 discloses diketopiperazines which inhibit apoptosis and are said to be useful for many diseases including multiple sclerosis.

[0021] WO0211676 discloses DKPs, including DA-DKP, which are useful as therapeutic compounds for a variety of diseases probably by binding to PAF and its receptor. Multiple sclerosis is not listed. These documents view DKPs, including DA-DKP, as therapeutic compounds but not therapeutic targets.

[0022] US4205057 discloses myelin encephalitogenic protein fragments in CSF as diagnostic marker for multiple sclerosis. >

[0023] Elevated levels of histidine-proline diketopiperazine (HP-DKP) have been detected in neurological disorders, including non-medicated schizophrenics and patients suffering from amyotrophic lateral sclerosis (Prasad, Peptides, 1995 16:1 pp. 151-164), and in patients with renal failure (Takahara et al., J. Clinical Endocrinol. Metab. 1983 56:2 pp. 312-319). HP-DKP may be derived from thyrorropin-releasing hormone (TRH) or its precursor (preproTRH) by unknown mechanisms and/or from other sources (Prasad, Peptides, 1995 16:1 pp. 151-164**)**.

SUMMARY OF THE INVENTION

[0024] The present invention is based on the discovery of objective biochemical markers useful for diagnosing and monitoring various diseases and medical conditions. The markers include diketopiperazines composed of the two N-terminal amino acids or the two C-terminal amino acids of disease-associated proteins. The term "protein" is used herein to mean protein, polypeptide, oligopeptide or peptide, and the term "disease-associated proteins" is used herein to mean proteins associated with specific diseases or conditions, including proteins from organs or tissues ("organ-specific" or "tissue-specific" proteins) affected by a disease or condition. The markers also include truncated disease-associated proteins from which the two N-terminal amino acids and/or the two C-terminal amino acids are missing. These markers are collectively referred to herein as "target markers".

[0025] Accordingly, the present invention provides a method of diagnosing or monitoring Multiple Sclerosis (MS) comprising the steps of obtaining a biological sample from a patient to be diagnosed or monitored, determining the quantity of a marker in said biological sample, wherein the marker is Asp-Ala DKP (DA-DKP), N-acetyl-Ala-Ser DKP (NAS-DKP), N-acetyl-Ala-phophorylated-Ser DKP, Arg-Arg DKP, Gln-Asn DKP and determining if the quantitiy of the marker in said biological sample is indicative of the presence, absence or status of MS. The target markers can be measured rapidly and conveniently, and these measurements provide objective evidence which will allow a reliable diagnosis to be made easily and quickly for multiple sclerosis

[0026] This method will be of great benefit, since it will allow treatment of Multiple Sclerosis (MS) to begin much earlier than is now possible. In addition, the measurement of the target markers will allow the status of Multiple Sclerosis (MS) Multiple Sclerosis (MS) to be monitored allowing for more effective treatment and for the evaluation of new drugs and other treatments.

[0027] An elevated level of DA-DKP and/or NAS-DKP in the biological sample is indicative of MS. Also, an elevated level of DA-DKP and/or NAS-DKP in the biological sample is indicative of active MS. Other MS diagnostic compounds are listed in Tables 1 and 2.

[0028] The invention also provides novel binding partners having specificity for the diketopiperazines. The binding partners are antibodies and/or aptamers that specifically recognize the diketopiperazines of the present invention. Such binding partners can be used in the methods of the present invention. Compositions and kits containing the novel binding partners are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029]

Figure 1: Printout from a mass spectrometer. The sample was recombinant beta-human chorionic gonadotropin processed by liquid chromatography followed by mass spectrometry.

Figure 2: Printout from a mass spectrometer. The sample was a plasma sample from a pregnant woman (patient 4) processed by liquid chromatography followed by mass spectrometry.

Figure 3: Printout from a mass spectrometer. The sample was recombinant erythropoietin processed by liquid chromatography followed by mass spectrometry.

Figure 4: Printout from a mass spectrometer. The sample was a plasma sample from a pregnant woman (patient 4) processed by liquid chromatography followed by mass spectrometry.

Figure 5: A clustering dendogram.

DETAILED DESCRIPTION OF THE PRESENTLY

PREFERRED EMBODIMENTS OF THE INVENTION

[0030] The present disclosure generally provides methods of diagnosing and monitoring diseases, conditions and disorders by quantitating markers for the diseases and conditions.

[0031] In one embodiment, the present disclosure provides methods of diagnosing and monitoring diseases or conditions characterized by the degradation of disease-associated proteins. The degradation products include diketopiperazines composed of the two N-terminal amino acids or the two C-terminal amino acids and the corresponding truncated disease-associated proteins lacking such terminal amino acids. Accordingly, the present invention is based on the discovery that these degradation products are useful markers for diagnosing and monitoring multiple Sclerosis (MS) as defined in the appended claims.

[0032] As noted above, the term "disease-associated proteins" is used herein to mean proteins associated with specific diseases, conditions or disorders, including proteins from organs or tissues ("organ-specific" or "tissue-specific" proteins) affected by a disease, condition or disorder. Examples of disease-associated proteins and their corresponding diseases and conditions are listed in Tables 1 and 2. Those skilled in the art can readily determine, without undue experimentation, other disease-associated proteins, their corresponding diseases or conditions, and useful markers based on the guidance provided herein.

[0033] The target markers quantified in the methods of this embodiment are formed by the degradation of the disease-associated proteins. It is believed that this degradation occurs in diseases or conditions involving or caused by acidosis, reactive oxygen species (ROS), inflammation, and/or conditions which cause the protonation of the N-terminal or C-terminal amino acids of the disease-associated proteins, such as the binding of certain ligands to the N-terminal or C-terminal amino acid. Diketopiperazines can also be formed *in vivo* due to the action of certain enzymes (*e.g.*, dipeptidyl peptidases or carboxypeptidases), and the activity of these enzymes may be altered in certain diseases, conditions and disorders. Dipeptidyl peptidases are amino peptidases which cleave the two amino acids of the N-termini of proteins with some specificity, while carboxypeptidases cleave amino acids from the C-termini of proteins: The placenta, for example, is rich in dipeptidyl peptidase IV. After the cleavage, or under specific conditions, the enzymes may be responsible for cyclization, as well as cleavage, of the amino acids. Alternatively, the second step (cyclization) may be non-enzymatic and may require the protonation of the N-terminus or C-terminus. Thus, the markers useful in the present invention include diketopiperazines composed of two amino acids from either terminal end of a disease-associated protein and the truncated disease-associated proteins without the two N-terminal and/or the two C-terminal amino acids.

[0034] As used herein, "X-Y DKP" or "X-Y-DKP" means a diketopiperazine (cyclic dipeptide) composed of two amino acids, X and Y, wherein X and Y are the two N-terminal or the two C-terminal amino acids of a disease-associated protein. X and Y may be the same or different and each may be any amino acid, including any post-translationally modified amino acid. Notwithstanding the foregoing, X-Y DKP may not be His-Pro DKP when a single diketopiperazine is the only marker measured. Table 3 lists the conventional three-letter and single-letter abbreviations for each amino acid. Post-translational modifications of amino acids are well known and include phosphorylation, acylation, cysteinylation, nitrosylation, and glycosylation.

[0035] Examples of diketopiperazines useful as markers in the present disclosure are listed in Tables 1 and 2 along with their corresponding diseases and disease-associated proteins. Those skilled in the art can readily identify, without undue experimentation, other diketopiperazines derived from the two N-terminus amino acids or the two C-terminus amino acids of a disease-associated protein that can be used as target markers of various diseases and conditions. The markers in the invention are defined in the appended claims.

TABLE 1

Disease	Protein	N-terminal DKP	MW
Multiple Sclerosis	Myelin basic protein	N-acetyl-Ala-	280

(continued)

Disease	Protein	N-terminal DKP	MW
	(MBP) MBP Beta-amyloid	phospho-Ser N-acetyl-Ala-Ser Asp-Ala	 200 186
Rheumatoid Arthritis	Rh Factor	Glu-Ile	242.3
ARDS*, Cystic Fibrosis	Pulmonary surfactant-associated proteins A, B and D	(A) Glu-Val (B) Phe-Pro (D)Ala-Glu	2228.24
Diabetes Mellitus	Insulin	Phe-Val Gly-Ile	246.34 170.24
Alzheimer's disease	Beta-amyloid tau protein	Asp-Ala Met-Ala	186.15
Parkinson's disease	alpha-synuclein	Met-Asp Glu-Lys	246.31 257.38
Inflammation (general)**	Albumin C-reactive protein Interleukin 8	Asp-Ala Gln-Thr Ala-Val	186.15 229.23 170.2
Ischemia (general)	Albumin	Asp-Ala	186.15
Cerebral Ischemia	S 100 family of proteins	Many	Many
Placental Ischemia	Beta-chorionic gonadotropin Fetal erythropoietin Pregnancy-associated protein A	Gly-Leu Ala-Pro Glu-Ala	170.24 168.18
Myocardial Infarction	Myoglobin Troponin I	Gly-Leu Pro-Glu	170.24 226.22
Prostate Cancer	Prostate Specific Antigen (PSA)	Lys-Ser Ile-Val	215.28
Pancreatitis	Amylase Lipase	Gln-Tyr Lys-Glu	291.3 257.28
Emphysema	alphal-antitrypsyn	Glu-Asp	244.23
Renal Disease, Cancer, Chemotherapy	Erythropoietin	Ala-Pro	168.18
Sepsis	Activated protein C	Ala-Asn	185.17
Hemoglobinopathies, Amemias	Tethal Chain Zeta Chain Alpha Chain Beta Chain Delta Chain Epsilon Chain Gamma AG	Ala-Leu Ser-Leu Val-Leu Val-His Val-His Val-His Gly-His	199.24 215.24 227.3 236.27 236.27 236.27 194.19
Congestive heart failure	Brain natriuretic peptide	His-Pro Ser-Pro	234.25 184.18

TABLE 2

Disease	Protein	C-terminal DKP	MW
Multiple Sclerosis	Myelin basic protein (MBP) Beta-amyloid	Arg-Arg Gln-Asn	312.36 242.23
Rheumatoid Arthritis	Rh Factor	Lys-Arg	284.35
ARDS*,	Pulmonary	(A) Glu-Phe	276.28
Cystic Fibrosis	surfactant-associated proteins A, B and D	(B) Ser-Met (D) Glu-Phe	218.26 276.28
Diabetes Mellitus	Insulin	Cys-Asn Lys-Ala	217.24 199.24
Alzheimer's disease	Beta-amyloid tau protein	Gln-Asn Gly-Leu	242.23 170.21
Parkinson's disease	alpha-synuclein	Ala-Ala Ala-Ala	142.14 142.14
Inflammation (general)**	Albumin C-reactive protein Interleukin 8	Gly-Leu Trp-Pro Asn-Ser	170.21 283.32 201.17
Ischemia (general)	Albumin	Gly-Leu	170.21
Cerebral Ischemia	S 100 family of proteins	Many	Many
Placental Ischemia	Beta-chorionic gonadotropin Fetal erythropoietin Pregnancy-associated protein A	Leu-Pro Asp-Arg His-Gly	210.27 271.26 194.19
Myocardial Infarction	Myoglobin Troponin I	Gln-Gly Glu-Ser	185.18 216.18
Prostate Cancer	Prostate Specific Antigen (PSA)	Asn-Pro Asn-Pro	211.21 211.21
Pancreatitis	Amylase Lipase	Lys-Leu Pro-Cys	241.33 200.25
Emphysema	alphal-antitrypsyn	Asn-Lys	256.3
Renal Disease, Cancer, Chemotherapy	Erythropoietin	Asp-Arg	271.26
Sepsis	Activated protein C	Ala-Pro	168.18
Hemoglobinopathies, Amemias	Tethal Chain Zeta Chain Alpha Chain Beta Chain Delta Chain Epsilon Chain Gamma AG		
Congestive heart failure	Brain natriuretic	Arg-His	293.32

(continued)

Disease	Protein	C-terminal DKP	MW
	peptide		
*ARDS = acute respiratory distress syndrome. **Asp-Ala diketopiperazine (DA-DKP) and/or Gly-Leu diketopiperazine (GL-DKP) derived from albumin, a circulating protein, will be general markers of inflammation. Other diketopiperazines derived from disease-associated proteins, including those found in specific organs or tissues, will be markers of inflammation in those organs and tissues or associated with those diseases and conditions.			

TABLE 3

Amino Acid	Three-Letter abbreviation	Single-Letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Gluatmine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0036] Other useful target markers formed by the degradation of the disease-associated protein are referred to as "truncated disease-associated proteins." As noted previously, these truncated disease-associated proteins lack the two N-terminal amino acids and/or the two C-terminal amino acids and, therefore, can be utilized as target markers in the present methods. However, the truncated disease-associated protein may not be human serum albumin lacking the two N-terminal amino acids. Truncated disease-associated proteins include, for example, myelin basic protein missing the amino acids N-acetyl-Ala and Scr from the N-terminus and beta-amyloid missing the amino acids Asp and Ala from the N-terminus, both of which are useful target markers of multiple sclerosis. Truncated beta-amyloid missing the two N-terminal amino acids Asp and Ala and truncated tau protein missing the two C-terminal amino acids Gly and Leu are examples of target markers for Alzheimer's disease. Those skilled in the art can readily identify other useful truncated disease-associated proteins as target markers of various diseases and conditions.

[0037] In the present invention, the methods comprise:

(a) determining the quantity of one or more target markers of the disease or condition in a biological sample obtained from a patient to be diagnosed or monitored; wherein the marker is Asp-Ala DKP (DA-DKP), N-acetyl-Ala-Ser DKP (NAS-DKP), N-acetyl-Ala-phophorylated-Ser DKP, Arg-Arg DKP, Gln-Asn DKP and

(b) determining if the quantity of the target marker is indicative of the presence, absence or status of Multiple Sclerosis (MS).

[0038] In the methods, the target markers can be quantified in any suitable biological sample derived from the patient to be diagnosed or monitored. Biological samples include suitable body fluids, such as serum, plasma, blood, urine, saliva, cerebrospinal fluid, tears, semen, vaginal secretions, amniotic fluid and cord blood. Also, lavages, tissue homogenates and cell lysates can be utilized and, as used herein, biological samples include such preparations.

[0039] The biological samples can be taken from a patient. The term "patient" includes any animal, preferably mammals, and most preferably humans. Those skilled in the art can readily determine appropriate diseases or conditions and their corresponding target markers for a particular patient.

[0040] The quantity of the target marker can be measured by any means known to those skilled in the art, including, for example, by mass spectrometry, immunoassays, chemical assays, sensitive liquid chromatography without mass spectrometry, and a variety of direct and indirect photometric techniques. For instance, a variety of analytical methods can be used to quantitate the target marker by mass spectrometry. Generally, the marker of interest can be isolated from the biological sample by a suitable technique, such as liquid chromatography or two-dimensional gel electrophoresis. Then the target marker can be quantitated by any mass spectrometry detection method, such as electrospray ionization mass spectrometry, liquid chromatography tandem mass spectrometry (LC-MS), matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), MALDI time-of-flight MS (MALDI-TOF-MS), etc. See, *e.g.,* Lim et al., Analytical Biochemistry, 295: 45-56 (2001). The target markers can be quantitated using pure marker standards of known quantity or by comparison to the same target markers in the same type of biological samples from normal controls.

[0041] Immunoassays are preferably used to quantitate the target markers. Immunoassays employ one or more binding partners. A "binding partner" is any compound or molecule capable of specifically binding to a target marker. As used herein, "specifically" means the binding partner binds to the target marker selectively in the presence of other compounds. Binding partners are preferably antibodies, aptamers, lectins and other molecules that can specifically bind to the target marker. Such binding partners can be used separately or in combination (*e.g.*, antibodies can be used in combination with aptamers). Suitable binding partners are described in the appended claims as a further embodiment of the present invention.

[0042] Those skilled in the art can readily determine immunoassay formats suitable for use in the methods of the present invention. Such immunoassays include homogeneous assays, heterogeneous assays, enzyme immunoassays (*e.g.*, ELISA), competitive assays, immunometric (sandwich) assays, turbidimetric assays, nephelometric assays, and the like. The immunoassays can be performed manually or with an automated analyzer.

[0043] In a preferred enzyme immunoassay, a binding partner specific for the target marker is immobilized on a solid substrate. Suitable solid substrates are well known and include, for example, glass, polystyrene, polypropylene, polyethylene, nylon, polyacrylamide and agarose. The biological sample is contacted with the immobilized binding partner. After washing, the target marker bound to the solid substrate by the bound binding partner is reacted with a second binding partner (*e.g.* a second antibody or a mixture of antibodies) specific for a known epitope on the target marker. The second binding partner can be labeled to quantitate the target marker or a labeled third binding partner or other compound (*e.g.*, protein A or streptavidin) can be used to quantitate the marker.

[0044] As an alternative, the target marker can first be separated from the other constituents of the biological sample by, *e.g.*, affinity chromatography. For affinity chromatography, antibodies specific for the target marker are attached to a solid surface (*e.g.*, beads in a column) and used to bind the target marker in the sample. After washing the solid surface, the target markers are eluted and measured (*e.g.*, by one of the methods described above, by measuring the absorbance at 280 nm or by any another method known to those skilled in the art).

[0045] Suitable labels for any of the binding partners (*e.g.* primary, secondary or third antibody) are well known in the art. Such labels include: (i) enzymes (*e.g.*, horseradish peroxidase, malate dehyrogenase, staphylococcal nuclease, delta-5-steriod isomerase, yeast alcohol dehydrogenase, alphaglycerophosphate dehydrogenase, triose phosphate isomerase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galatosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholine esterase); (ii) fluorophores (*e.g.*, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phtaldehyde and fluorescamine); (iii) radionucleotides (*e.g.*, indium-111, technetium-99m, iodine-125, gallium-67 and gallium-68); (iv) bioluminescent labels (*e.g.*, luciferin, luciferase and aequorin); (v) chemiluminescent labels (*e.g.*, luminol, isoluminol, aromatic acridinium ester, imadazole, acridinium salt and oxalate ester); (vi) colorimetric labels; (vii) metal colloid labels; (viii) latex and silica particles with dyes incorporated into them; (ix) dyes; and (x) affinity labels (*e.g.*, biotin). The binding and detection of these labels can be done using techniques known to those skilled in the art.

[0046] It is then determined if the quantity(ies) of the target marker(s) in the biological sample is(are) indicative of the presence, absence or status of a disease or condition. This is accomplished using any of a variety of well known methods of statistical analysis. For instance, a clustering technique, such as the one exemplified in Example 2, can be used. Alternatively, the determination can be accomplished by comparing the quantity(ies) of the target marker(s) in the sample to the quantity(ies) of the target marker(s) in normal patients. "Normal patients" are those not suffering from the particular disease or condition to be diagnosed or monitored. For instance, the amount of a target marker can be compared to a normal range. This normal range is found by determining the amount of the marker in a large number of samples from normal individuals by the same method (*i.e.*, same type of biological sample, same steps, same reagents, same conditions) as used in assaying the patient sample. If the amount of target marker is outside the normal range, then the presence of the disease or condition is indicated. Alternatively, the amount of a target marker can be compared with a cut-off value that is indicative of the disease or condition. The cut-off value can be determined by testing a large number of samples from normal individuals and from patients known to be suffering from a particular disease or condition of interest. If the amount of target marker exceeds the cut-off, then the disease or condition is indicated. Further, the amount of a target marker and/or the presence of two or more target markers outside their normal ranges or which exceed their cutoffs may also be indicative of the status of disease or condition. In analyzing data, including determining a normal range or cut-off value, standard statistical methods well known in the art can be used. Finally, as can be appreciated, the normal ranges and the cut-off values can be expressed in the units of detection (*e.g.*, levels of absorbance or of fluorescence) as a matter of convenience and ease of making the correlation.

[0047] For example, as noted above, the measurement of DA-DKP can be used in the diagnosis or monitoring of MS. DA-KDP levels in normal human patients is in the range of about 50-100 ng/ml. Those skilled in the art will be able to readily determine when the level of DA-DKP is significantly elevated to indicate multiple sclerosis using any of the methods disclosed herein or other known statistical methods.

[0048] The above methods of the present disclosure can be used to diagnose or monitor a number of diseases and conditions. These diseases and conditions include, but not limited to, those identified in Tables 1 and 2.

[0049] In a further embodiment, the present disclosure provides objective biochemical markers useful for the diagnosis and monitoring of multiple sclerosis (MS) in patients. In particular, the following markers have been identified in plasma samples by liquid chromatography followed by mass spectrometry (LC-MS):

1. First, a compound of mass 175 (actual mass of 176) has been found to be missing from the plasma of MS patients as compared to plasma samples from normal humans.
2. A compound of mass 145 (actual mass of 146) has also been found to be missing from the plasma of MS patients as compared to plasma samples from normal humans.
3. A compound of mass 185 (actual mass 186) has been found to be significantly elevated in the plasma of MS patients who have active disease as compared to plasma samples from normal humans and from MS patients whose disease is not active. This compound has been identified as the cyclic dipeptide aspartic acid-alanine diketopiperazine (DA-DKP). It is interesting to note that this compound has been shown to inhibit platelet activating factor and to inhibit the production and/or release of interleukin- 8 (see PCT application WO 02/11676).
4. A compound of mass 199 (actual mass 200) has been found to be significantly elevated in the plasma of MS patients who have active disease as compared to plasma samples from normal humans and from MS patients whose disease is not active. This compound has been identified as N-acetyl-alanine-serine diketopiperazine (NAS-DKP).

[0050] Thus, the absence of one or both of the compounds of masses 175 and 145 from a plasma sample indicates that the patient has MS. A significantly elevated level of one or both of the diketopiperazines of masses 185 and 199 indicates that the patients are suffering from active MS. MS diagnostic compounds include, but are not limited to, all of these compounds and the diketopiperazines and truncated disease-associated proteins of Tables 1 and 2.

[0051] "Active MS" is used to mean the period when new, additional or worsening clinical manifestations occur (an attack, exacerbation, flare or relapse). It is usually associated with increased myelin/neuron destruction, elevated white blood cells (>4/hpf) and IgG synthesis rate (>9) in the cerebrospinal fluid, MRI demyelination plaques, and "black holes" which represent neuronal loss.

[0052] In yet another embodiment, the present disclosure provides objective biochemical markers useful for the diagnosis or monitoring of Alzheimer's disease. The present disclosure provides methods of diagnosing or monitoring Alzheimer's disease using an Alzheimer's diagnostic compound. The methods are accomplished by obtaining a biological sample from a patient to be diagnosed or monitored and determining the amount of an Alzheimer's diagnostic compound in the biological sample. Alzheimer's diagnostic compounds include, for example: (i) a compound having a mass of about 175 as determined by liquid chromatography and mass spectrometry; and (ii) the diketopiperazine derived from beta-amyloid which is Asp-Ala DKP (MW 186.15). Both diagnostic compounds have been found elevated in the plasma of Alzheimer's patients and are considered diagnostic of the disease. Other Alzheimer's diagnostic compounds include the diketopiperazines and truncated disease-associated proteins of Tables 1 and 2.

[0053] In a further embodiment, the present disclosure provides methods for the diagnosis or monitoring of placental ischemia in pregnant patients. These methods comprise obtaining a biological sample from a pregnant patient and measuring the amount of a placental ischemia diagnostic compound, including those derived from pregnancy-associated proteins, in the biological sample. Examples of placental ischemia diagnostic compounds useful in the methods include, for example: (i) Gly-Leu diketopiperazine (GL-DKP) derived from beta-human chlorionic gonadotropin; and (ii) Ala-Pro diketopiperazine (AP-DKP) derived from fetal erythropoietin. Other placental ischemia diagnostic compounds include the diketopiperazines and truncated disease-associated proteins of Tables 1 and 2.

[0054] Those skilled in the art will be able to readily isolate and determine the chemical composition of those compounds identified above only by their masses. Once their chemical compositions are known, they can be assayed by methods other than mass spectrometry, including those methods described above, preferably by means of an immunoassay.

[0055] In yet another embodiment, the present disclosure provides binding partners useful in the immunoassays described above. Binding partners include antibodies, antiserum or a purified fraction thereof, aptamers and other compounds capable of specifically binding to a target marker. Suitable antibodies include polyclonal antibodies, monoclonal antibodies, bispecific antibodies, humanized antibodies, chimeric antibodies, single-chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by an Fab expression library, epitope-binding fragments of any of the foregoing, and complementarity determining regions (CDRs). Antibodies suitable for use in the invention can be prepared by known methods. Particularly suitable antibodies are monoclonal antibodies having specificity for the diketopiperazines of the present invention as described in the appended claims. Since the diketopiperazines are small compounds, they will preferably be attached to an immunogenic carrier molecule for use as an immunogen to prepare antibodies specific to them. Suitable carrier molecules (*e.g.*, KLH) and methods of attaching molecules to them are well known in the art. The immunogen can be used to produce monoclonal antibodies using fusion procedures of Kohler and Milstein, Nature 1975 256 pp.495-497, with modifications known to those skilled in the art. The term "isolated" used in connection with binding partner means the binding partner is not within the milieu of its natural environment if found in nature and is not meant to indicate any level of purity of the binding partner.

[0056] Aptamers can be used in place of, or in combination with, the antibodies in any of the above described immunoassays. Aptamers are oligonucleotides that are specific for proteins, peptides, derivatives of proteins and peptides, inorganic molecules and other non-nucteotide molecules. See, *e.g.*, PCT applications WO 00/070329, WO 01/79562 and WO 99/54506, and U.S. Patent No. 5,756,291 . Aptamers suitable for use in the present invention as described in the appended claims can be prepared using the methods described in these references. Briefly, a heterogeneous population of oligonucleotides of random sequences is synthesized, and a marker of the invention is mixed with the heterogeneous population of oligonucleotides. Complexes are formed with some, but not all, of the sequences present in the oligonucleotide population. The complexes are isolated, and the oligonucleotides recovered and amplified (*e.g.*, by PCR). The resulting mixture of oligonucleotides can be used as the starting material for another round of complexation, isolation and amplification, and the process will typically be repeated several times until an aptamer of satisfactory specificity is obtained and/or until a consensus aptamer sequence is identified. Suitable labels for aptamers include dyes, enzymes, radioactive labels, etc.

[0057] The present invention further provides compositions containing the binding partners as described in the appended claims in a physiologically-acceptable carrier. Such physiologically-acceptable carriers are well known in the art and include, for example, aqueous solutions such as bicarbonate buffers, phosphate buffers, physiological saline, Ringer's solution and the like.

[0058] The invention also provides kits for quantifying the target markers as described in the appended claims. Such kits optionally contain various reagents useful for conducting the methods of the present invention, including one or more binding partners specific for a target marker, a labeled component useful for detecting the target marker, buffers, diluents, standards, controls, etc. The kits can also contain bottles, vials, tubes, syringes, microtiter plates or other solid substrates, instructions and the like.

[0059] The following Examples are intended to illustrate the embodiments of the invention and the present disclosure and are not intended to limit the invention.

EXAMPLES

EXAMPLE 1: Diagnosis of placental ischemia, not part of the invention

[0060] The presence of several diketopiperazines has been detected in maternal plasma. Of particular interest are the ones derived from the N-termini of β-human chorionic gonadotropin (βHCG) and fetal erythropoietin. These are glycine-leucine diketopiperazine **(GL-DKP)** and alanine-proline diketopiperazine **(AP-DKP),** respectively. AP-DKP, in particular, is elevated in FGR pregnancy due to elevation of fetal erythropoietin in FGR (Teramo, et al., Acta Obstet. Gynecol. Scared. 2002. 83(1): p. 245-51; Jazayeri et al., Am. J. Obstel. Gynecol., 2000. 183(1): p. 188-90; Jazayeri et al., J. Perinatol., 1999. 19(4): p. 255-9) and its specific degradation in acidic conditions (protonation of the N-terminal amino acid (Goolcharran and Borchardt, J. Pharm. Sci., 1998. 87(3): p. 283-8) and the relative importance for proline in position 2 of the primary amino acid sequence) to yield AP-DKP.

[0061] Subjects for the study were selected from patients referred to a Maternal-Fetal Medicine (MFM) practice with complicated pregnancies. Inclusion criteria for the study were:

Estimated fetal weight < 10th percentile for gestational age by ultrasound in addition to:

- an amniotic fluid index (AFI) < 8 or,
- a ratio of blood flow velocity during systole to diastole (S/D) in the umbilical artery as measured with pulse-wave Doppler > 3 or,
- preeclampsia, as defined by standard clinical criteria.

[0062] There were 12 patients in the study group, including 11 singletons and one twin gestation. There were 5 patients in the control group including 1 twin gestation. Gestational ages in the study group at time of delivery were between 26.3-38 weeks with an average gestational age of 30.2 weeks versus 38 weeks in the control group. Average birth weights were 1016 grams in the study groups versus 3114 grams in the control group. Birth weight percentages for the study group averaged < 10% versus 43% in the control group. Umbilical artery Doppler flow studies were obtained in 10 of the 12 study patients; of these, all were abnormal, with 2 patients having reversed end-diastolic flow, 6 having absent end-diastolic flow, and 2 having an S/D ratio > 3.0. Nine of the 12 study patients had preeclampsia. Two of the 12 study patients had HELLP syndrome.

[0063] Recombinant βHCG (Sigma) was incubated in phosphate buffer 0.1M, pH 7.4 at 60°C for 12 hours and analyzed for the presence of GL-DKP (MW 170.21) by liquid chromatography (LC) followed by ESI+ mass spectrometry (ESI+/MS). The results are presented in Figure 1.

[0064] Similarly, recombinant erythropoietin (Amgen) was incubated in phosphate buffer 0.1M, pH 7.4 at 60°C for 12 hours and analyzed for the presence of AP-DKP (MW 168.18) by LC and ESI-/MS. The results are presented in Figure 3.

[0065] Plasma samples were taken from study group patients and the control group patients and processed by LC followed by ESI/MS. The results for one study group patient (patient 4) are presented in Figures 2 and 4. As can be seen, GL-DKP (derived from βHCG) and AP-DKP (derived from fetal erythropoietin) were detected.

EXAMPLE 2: Classification of MS Patients Using Liquid Chromatography - Mass Spectrometry and Clustering

[0066] A novel method of determining the Multiple Sclerosis (MS) status of patients is presented here along with some results of a small test set of both MS and normal patients. In the present method, blood samples were collected from both normal and MS patients and analyzed by a liquid chromatography - mass spectrometry (LC-MS) method to determine the concentration of several putative MS markers. The resulting data were analyzed by a mathematical clustering technique that finds natural groupings within the data to see whether there were any simple relationships between the levels of these putative markers and the presence, absence or status of MS.

Patients

[0067] Patients with MS were diagnosed by accepted clinical and laboratory standards. Neurological signs and symptoms, magnetic resonance imaging evidence of demyelination, presence of oligoclonal bands in cerebrospinal fluid, white cell enumeration and IgG synthesis rates were used to make the diagnosis. Active disease was defined based on the above in the presence of acute or progressive neurological manifestations.

Sample Preparation

[0068] Blood samples were collected in heparinized tubes. The blood samples were separated via centrifugation into plasma and red cells. The red cells were discarded, and the plasma was further refined by passing it through a size exclusion filter (Centricon 3) to remove all components that were greater than 3,000 daltons. The resulting filtrates were analyzed immediately or frozen for later analysis.

LC-MS Method

[0069] The samples were run on an HPLC (a Waters 2975 system) to separate the various components. The column used was an Amersham mono-Q anion exchange. The mobile phase was a 50 mM solution of ammonium acetate, pH 6.7, run at I ml/min. The flow was split 4:1 post column leaving a 250 ul/min stream which was routed to a Micromass LCT mass spectrometer operated in negative electrospray ionization (ESI-) mode using a cone voltage of 20v. Because of the high flow rate and high aqueous content of the solvent, the desolvation temperature was set to 400°C. Standards of DA-DKP and EA-DKP were run with each set of data to calibrate for transient differences in instrument sensitivity. The standard concentrations used are 500, 100, 20, and 4 ng/ml of each DKP. Detection of the DKPs by the mass spectrometer was found to be linear in this range of concentrations (r2>0.998).

Data Preparation

[0070] While there are no calibrants for some of the putative markers described below, it was assumed that the sensitivity of the instrument was linear across the spectrum and, thus, all masses for which there was no calibrant were normalized to the level of 500 ng/ml DA-DKP.

Mathematical analysis

[0071] Clustering is a classification technique that identifies groups of similar objects where similarity is derived solely on the basis of the variables that describe the data. Ideally, the groups are formed in such a way that objects within a group are similar to each other, while objects in different groups as are as dissimilar as possible.

[0072] When one tries to cluster raw data from experimental data whose variables are poorly scaled, components with large magnitude will dominate any distance metric, resulting in a disproportionate weighting of those variables. Since one has no *a priori* knowledge of each variable's importance, one scales to give equal weight to each of the variables. Dimensional scaling is employed so each variable is shifted and scaled. As a result, the means are zero and the variances are-equal.

[0073] Often, experiments generate high-dimensional data sets which may have strong dependencies. In order to maximize the likelihood of a nontrivial classification, one wishes to minimize the number of dimensions by extracting the most relevant information from the data while minimizing noise. Methods of feature extraction include wavelet decomposition, Fourier transformation, factor analysis and independent component analysis.

[0074] In this work, feature extraction was performed using a variant of factor analysis called principal component analysis (PCA). In PCA, the data are represented as coefficients of the eigenvectors of the covariance matrix that describes the data. Moreover, the relative strengths of each of the eigenvectors (also known as principal components) are given by the eigenvalues. Eigenvectors with corresponding eigenvalues that are below some threshold can often be omitted as noise:

[0075] After choosing a set of 10 putative masses for analysis, the data were analyzed with a clustering toolset in Matlab written by Raphael Bar-Or, DMI BioSciences, Inc., Englewood, Colorado. Other suitable clustering software is available commercially. Trial and error analysis revealed that 2 masses of 185 and 199 appearing early in the runs had some power to separate the data into 2 groups, one of which is active MS and the other non-active MS and normals. In a subset of MS patients and normal patients, the settings of the clustering toolkit were optimized to achieve good separation between active MS and all other diagnoses. The settings for this analysis are given in Table 4.

[0076] Samples from a total of 37 patients were run. Of these, 24 were in cluster #1 (8 normals and 16 non-active MS), and 13 were in cluster #2 (active MS). There appear to be few, if any, misclassifications, and an inspection of the clustering dendrogram (Figure 5) reveals that the space is quite separable, meaning that there is sufficient space between the clusters so that this separation is not likely to be the product of chance alone. A small bootstrap (leave-one-out analysis) confirmed that the separation is indeed stable (93% by Rand's Statistic).

[0077] The groups found by the clustering method were used as a classifier. In this small data set, a sensitivity of 100% and a specificity of 84.6% for active MS were found.

[0078] The two masses of 185 and 199 were identified to be Asp-Ala DKP (DA-DKP) and N-acetyl-Ala-Ser DKP (NAS-DKP), respectively. These two DKPs are the degradation products of two important central nervous system proteins, namely beta amyloid and myelin basic protein (see Table 1).

[0079] It was noted that a marker labeled "175 @ 8.5 mins" appears to be deficient in all of the suspected MS patients and unusually high in Alzheimer's patients. A similar pattern was observed in another mass labeled "145 @ 12.7 mins". Addition of this variable to the clustering analysis would surely improve the separation, but data beyond 6 minutes is available for only a small subset of patients (only 14 samples were run for longer than 6 minutes, and only 10 of these had also been run for the shorter time). An analysis of this smaller group revealed that a simple threshold on the level of "175 @ 8.5 mins" was sufficient to quite accurately separate MS from normals and non-MS patients without distinguishing between the active and non-active forms. While there is insufficient data to conclude that this 175 marker is definitive, the evidence suggests that, together with the two markers (185 and 199) used in the above clustering analysis, there is a strong likelihood for an algorithm that can accurately separate MS patients from normals and non-MS patients and that the MS patients can be further categorized into active and non-active MS.

TABLE 4

```
%***************************************************************************************************
%**** EDIT THE VALUES IN THIS BLOCK ********************************************
%***************************************************************************************************
standout=[];
% elements to color differently so that they stand out
logdata=1;
% convert to log data
scaling=0;
% 1 = dimensional
scaling % 0 = no dimensional scaling
number of clusters =2;
% the number of desired clusters (should be =>2)
convert Pca=1;
% 1 = convert to pca space
% 0 = no conversion (original space)
keepvariation=0.95;
% the amount of variability to keep in the pca conversion
clusterAlgorithm=’hierarchical’;
% type of clustering desired options are:
% ’kplane’
% ’kmeans’
% ’kmedians’
% ’fuzzy_cmeans’
% ’hierarchical’
% ’gravity’
gravorder=15;
% only applicable if cluster type is gravity
gravtol=2e-3;
% only applicable if cluster type is gravity
addmasses=1;
% only applicable if cluster type is gravity
heirarchicalMetric=’Euclid’;
% n/a if clusteralgorithm is not ’hierarchical’ or ’permutation’
% options are:
% ’Euclid’=Euclidean distance (default)
% ’SEuclid’=Standardized Euclidean distance
% ’Mahal’=Mahalanobis distance
% ’CityBlock’=City Block metric
% ’Minkowski’=Minkowski metric
% ’Correlation’=1-Correlation coefficients

heirearchicalLinkage=’ward’;
%’single’=Shortest distance
%’complete’=Largest distance
%’average’=Average distance
%’centroid’=Centroid distance (approximate, computed using a formula that is exact if γ
contains Euclidean distances)
%’ward’=Incremental sum of squares

displayClusterInfo-1;
% 1 = display cluster info
```

(continued)

```
%**************************************************************************************************
%**** EDIT THE VALUES IN THIS BLOCK ********************************************
%**************************************************************************************************
% 0 = no cluster info display

displayScatterplot=1;
% 1 = display scatterplot of first three components
% 0 = no scatterplot

displayClusterGeneResponse=1;
% 1 = display gene response representation for each cluster
% 0 = no display
typeResponseRep='line';
% n/a if displayClusterGeneResponse is disabled
% are:

options % 'box'
% 'line'
% 'bar'
% 'area'

crossvalidation=0;
% leave one out cross-validation (can take a VERY long time)
% 1 = do cross validation
% 0 = no cross validation
```

EXAMPLE 3: Analysis Of An MS Patient Using Liquid Chromatography - Mass Spectrometry

[0080] A blood sample was collected from an MS patient with active MS and processed and analyzed by LC-MS as described in Example 2. The following DKP's were found: DA-DKP (from N-terminus of beta-amyloid), NAS-DKP (from N-terminus of myelin basic protein), N-acetyl-Ala Phospho-Ser DKP (from N-terminus of myelin basic protein), Gln-Asn DKP (from C-terminus of beta-amyloid) and Arg-Arg DKP (from C-terminus of myelin basic protein).

EXAMPLE 4: Diagnosis of Alzheimer's Disease, not part of invention

[0081] As noted in Example 2, a marker labeled "175 @ 8.5 mins" was found to be present in unusually high amounts in the plasma of Alzheimer's patients. It is expected that this marker will be useful in the diagnosis of Alzheimer's disease.

[0082] In addition, a marker at mass 186.15, which DA-DKP, has been found elevated in the plasma of Alzheimer's patients. It appears to be diagnostic of the disease.

[0083] Finally, another possible marker of mass 200 (actual mass 201) has been found. It has not yet been identified, but a likely candidate is NAS-DKP.

Claims

1. A method for diagnosing or monitoring Multiple Sclerosis (MS) comprising the steps of:

(a) determining the quantity of a marker in a biological sample obtained from a patient to be diagnosed or monitored, wherein the marker is:

(i) Asp-Ala DKP (DA-DKP);
(ii) N-acetyl-Ala-Ser DKP (NAS-DKP);

(iii) N-acetyl-Ala-phosphorylated-Ser DKP;
(iv) Arg-Arg DKP;
(v) Gln-Asn DKP; and

(b) determining if the quantity of the marker in said biological sample is indicative of the presence, absence or status of MS.

2. The method of Claim 1 wherein the quantity of both (i) and (ii) is determined or wherein the quantity of (i) to (v) is determined.

3. The method of Claim 1 or 2, wherein the quantity of a marker is determined by mass spectrometry, chemical assay or immunoassay.

4. The method of Claim 3, wherein the quantity of a marker is determined by immunoassay.

5. The method of Claim 4, wherein said immunoassay is conducted by using a binding partner specific for a marker, wherein the binding partner is an antibody or an aptamer.

6. The method of any preceding claim, wherein the biological sample is a body fluid.

7. The method of Claim 6, wherein the body fluid is serum, plasma, blood, urine, saliva, cerebrospinal fluid, tears, semen, vaginal secretion, amniotic fluid or cord blood.

8. The method of Claim 7, wherein the body fluid is plasma or serum.

9. The method of any preceding claim, wherein said patient is a human.

10. An isolated binding partner having specificity for a marker in a biological sample indicative of the presence, absence or status of MS, the marker selected from the groups (i) to (v) listed in Claim 1, wherein the binding partner is an antibody or an aptamer.

11. The isolated binding partner of Claim 10, wherein said binding partner is a monoclonal antibody.

12. The isolated binding partner of Claim 10, wherein said binding partner is an aptamer.

13. A composition comprising the binding partner of any one of Claims 10 to 12 in a physiologically-acceptable carrier.

14. A quantifying kit for quantifying a marker in a biological sample indicative of the presence, absence or status of MS comprising the binding partner of any one of Claims 10 to 12 and associated reagents.

Patentansprüche

1. Verfahren zum Diagnostizieren oder Überwachen von Multipler Sklerose (MS), umfassend die Schritte:

(a) Bestimmen der Menge eines Markers in einer biologischen Probe, die von einem Patienten erhalten wurde, bei dem eine Diagnose zu stellen ist oder der zu überwachen ist, wobei der Marker ist:

(i) Asp-Ala-DKP (DA-DKP);
(ii) N-Acetyl-Ala-Ser-DKP (NAS-DKP);
(iii) N-Acetyl-Ala-phosphoryliertes Ser-DKP;
(iv) Arg-Arg-DKP;
(v) Gln-Asn-DKP; und

(b) Bestimmen, ob die Menge des Markers in der biologischen Probe für das Vorliegen, das Nichtvorhandensein oder den Status von MS indikativ ist.

2. Verfahren gemäß Anspruch 1, wobei die Menge sowohl von (i) als auch (ii) bestimmt wird oder wobei die Menge von (i) bis (v) bestimmt wird.

3. Verfahren gemäß Anspruch 1 oder 2, wobei die Menge eines Markers durch Massenspektrometrie, chemischen Assay oder Immunoassay bestimmt wird.

4. Verfahren gemäß Anspruch 3, wobei die Menge eines Markers durch Immunoassay bestimmt wird.

5. Verfahren gemäß Anspruch 4, wobei der Immunoassay durchgeführt wird, indem ein für einen Marker spezifischer Bindungspartner verwendet wird, wobei der Bindungspartner ein Antikörper oder ein Aptamer ist.

6. Verfahren gemäß einem vorangehenden Anspruch, wobei die biologische Probe eine Körperflüssigkeit ist.

7. Verfahren gemäß Anspruch 6, wobei die Körperflüssigkeit Serum, Plasma, Blut, Urin, Speichel, Zerebrospinalflüssigkeit, Tränen, Sperma, Vaginalsekret, Fruchtwasser oder Nabelschnurblut ist.

8. Verfahren gemäß Anspruch 7, wobei die Körperflüssigkeit Plasma oder Serum ist.

9. Verfahren gemäß einem vorangehenden Anspruch, wobei der Patient ein Mensch ist.

10. Isolierter Bindungspartner, der Spezifität für einen Marker in einer biologischen Probe hat, welcher für das Vorliegen, das Nichtvorhandensein oder den Status von MS indikativ ist, wobei der Marker aus den Gruppen (i) bis (v), die in Anspruch 1 aufgelistet sind, ausgewählt ist, wobei der Bindungspartner ein Antikörper oder ein Aptamer ist.

11. Isolierter Bindungspartner gemäß Anspruch 10, wobei der Bindungspartner ein monoklonaler Antikörper ist.

12. Isolierter Bindungspartner gemäß Anspruch 10, wobei der Bindungspartner ein Aptamer ist.

13. Zusammensetzung, die den Bindungspartner gemäß einem der Ansprüche 10 bis 12 in einem physiologisch verträglichen Träger umfasst.

14. Quantifizierungs-Kit zur Quantifizierung eines Markers in einer biologischen Probe, der für das Vorliegen, das Nichtvorhandensein oder den Status von MS indikativ ist, umfassend den Bindungspartner gemäß einem der Ansprüche 10 bis 12 und assoziierte Reagenzien.

Revendications

1. Procédé de diagnostic ou de contrôle de la sclérose en plaques (SEP) comprenant les étapes consistant à :

(a) déterminer la quantité d'un marqueur dans un échantillon biologique obtenu chez un patient à diagnostiquer ou contrôler, où le marqueur est :

(i) Asp-Ala DKP (DA-DKP) ;
(ii) N-acétyl-Ala-Ser DKP (NAS-DKP) ;
(iii) N-acétyl-Ala-phosphoryle-Ser DKP ;
(iv) Arg-Arg DKP ;
(v) Gln-Asn DKP ; et

(b) déterminer si la quantité du marqueur dans ledit échantillon biologique indique la présence, l'absence ou l'état de SEP.

2. Procédé selon la revendication 1, dans lequel la quantité à la fois de (i) et (ii) est déterminée ou bien dans lequel la quantité de (i) à (v) est déterminée.

3. Procédé selon la revendication 1 ou 2, dans lequel la quantité d'un marqueur est déterminée par spectrométrie de masse, dosage chimique ou dosage immunologique.

4. Procédé selon la revendication 3, dans lequel la quantité d'un marqueur est déterminée par un dosage immunologique.

5. Procédé selon la revendication 4, dans lequel ledit dosage immunologique est conduit en utilisant un partenaire de liaison spécifique d'un marqueur, où le partenaire de liaison est un anticorps ou un aptamère.

6. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'échantillon biologique est un liquide corporel.

7. Procédé selon la revendication 6, dans lequel le liquide corporel est le sérum, le plasma, le sang, l'urine, la salive, le liquide céphalo-rachidien, les larmes, le sperme, les sécrétions vaginales, le liquide amniotique ou le sang du cordon.

8. Procédé selon la revendication 7, dans lequel le liquide corporel est le plasma ou le sérum.

9. Procédé selon l'une quelconque des revendications précédentes, où ledit patient est un être humain.

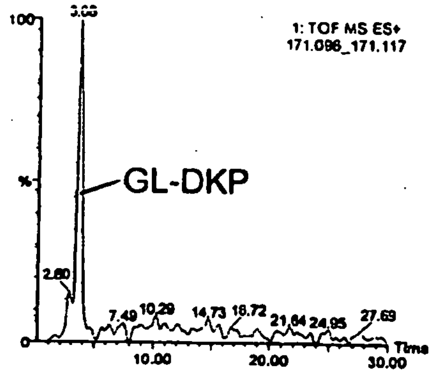
10. Partenaire de liaison isolé présentant une spécificité pour un marqueur dans un échantillon biologique indiquant la présence, l'absence ou l'état de SEP, le marqueur choisi dans les groupes (i) à (v) énoncés dans la revendication 1, où le partenaire de liaison est un anticorps ou un aptamère.

11. Partenaire de liaison isolé selon la revendication 10, où ledit partenaire de liaison est un anticorps monoclonal.

12. Partenaire de liaison isolé selon la revendication 10, où ledit partenaire de liaison est un aptamère.

13. Composition comprenant le partenaire de liaison selon l'une quelconque des revendications 10 à 12 dans un support physiologiquement acceptable.

14. Kit de quantification pour quantifier un marqueur dans un échantillon biologique indiquant la présence, l'absence ou l'état de SEP comprenant le partenaire de liaison selon l'une quelconque des revendications 10 à 12 et des réactifs associés.



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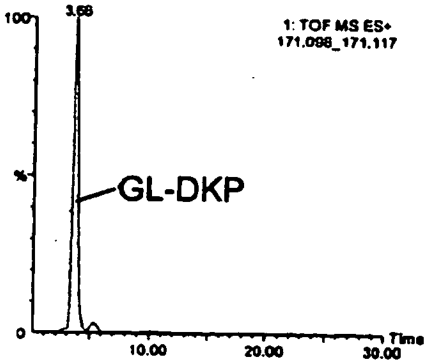

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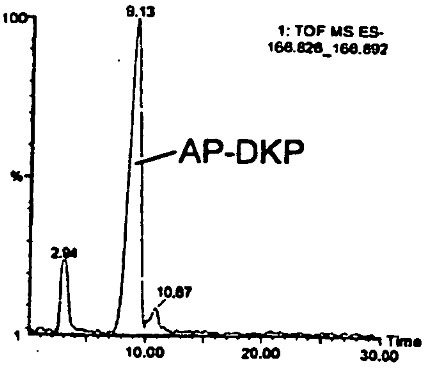

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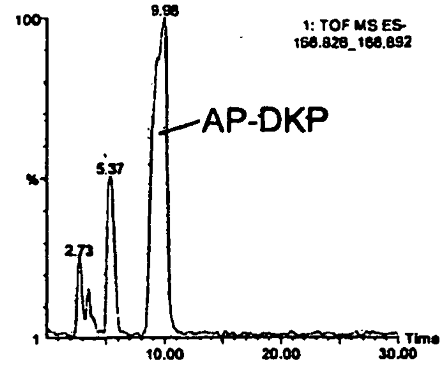

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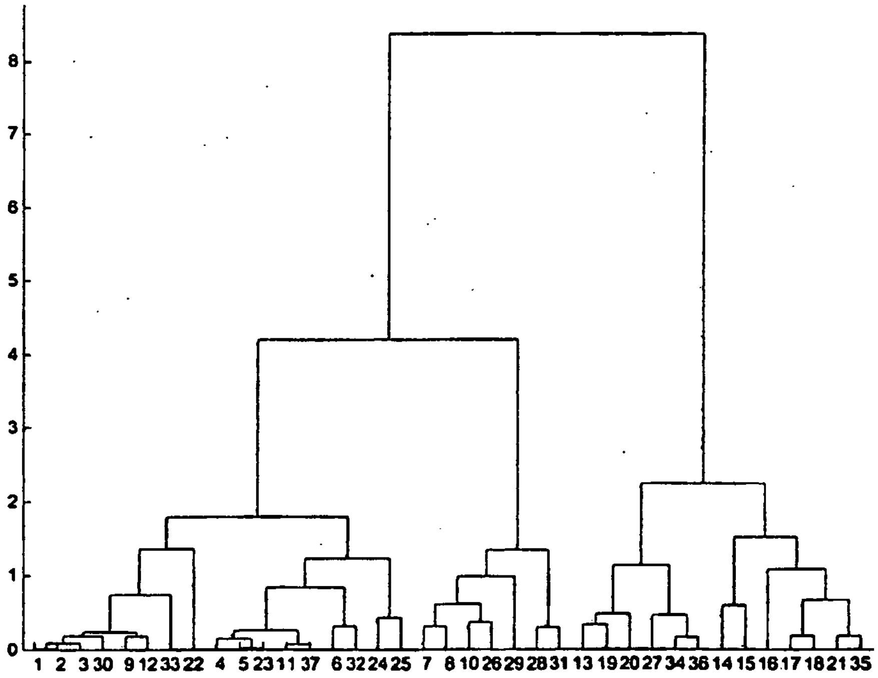

Figure 5

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专利名称(译)	疾病的诊断和监测		
公开(公告)号	EP1571970B1	公开(公告)日	2011-08-17
申请号	EP2003773099	申请日	2003-10-02
[标]申请(专利权)人(译)	DMI生物科学公司		
申请(专利权)人(译)	DMI BIOSCIENCES，INC.		
当前申请(专利权)人(译)	DMI BIOSCIENCES，INC.		
[标]发明人	BAR OR DAVID BAR OR RAPHAEL		
发明人	BAR-OR, DAVID BAR-OR, RAPHAEL		
IPC分类号	G01N33/53 G01N33/576 A01N1/00 A01N1/02 A01N37/18 A61K38/00 A61B A61B1/00 A61K39/395 C07K16/18 G01N33/00 G01N33/48 G01N33/483 G01N33/50 G01N33/68		
CPC分类号	G01N33/6848 G01N33/6842 G01N33/6896 G01N2800/285		
优先权	60/415908 2002-10-02 US 60/489039 2003-07-21 US 60/503185 2003-09-15 US		
其他公开文献	EP1571970A2 EP1571970A3 EP1571970A4		
外部链接	Espacenet		

摘要(译)

本发明涉及通过定量标记来诊断和监测疾病和病症，所述标记包括疾病相关蛋白的降解产物，例如由这些蛋白质的两个N-末端氨基酸或两个C-末端氨基酸组成的二酮哌嗪。该方法可用于诊断或监测各种疾病，包括多发性硬化症，阿尔茨海默病和局部缺血。本发明进一步提供了对标记和组合物特异的结合配偶体和用于实施本发明方法的试剂盒。

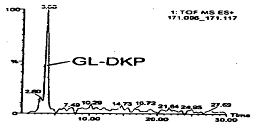
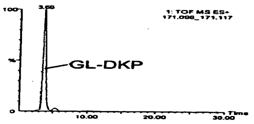


Figure 1.



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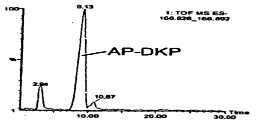

Figure 3.