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(54) **MULTIPLE FORMS OF ALZHEIMER'S DISEASE BASED ON DIFFERENCES IN CONCENTRATIONS OF PROTEIN BIOMARKERS IN BLOOD SERUM**

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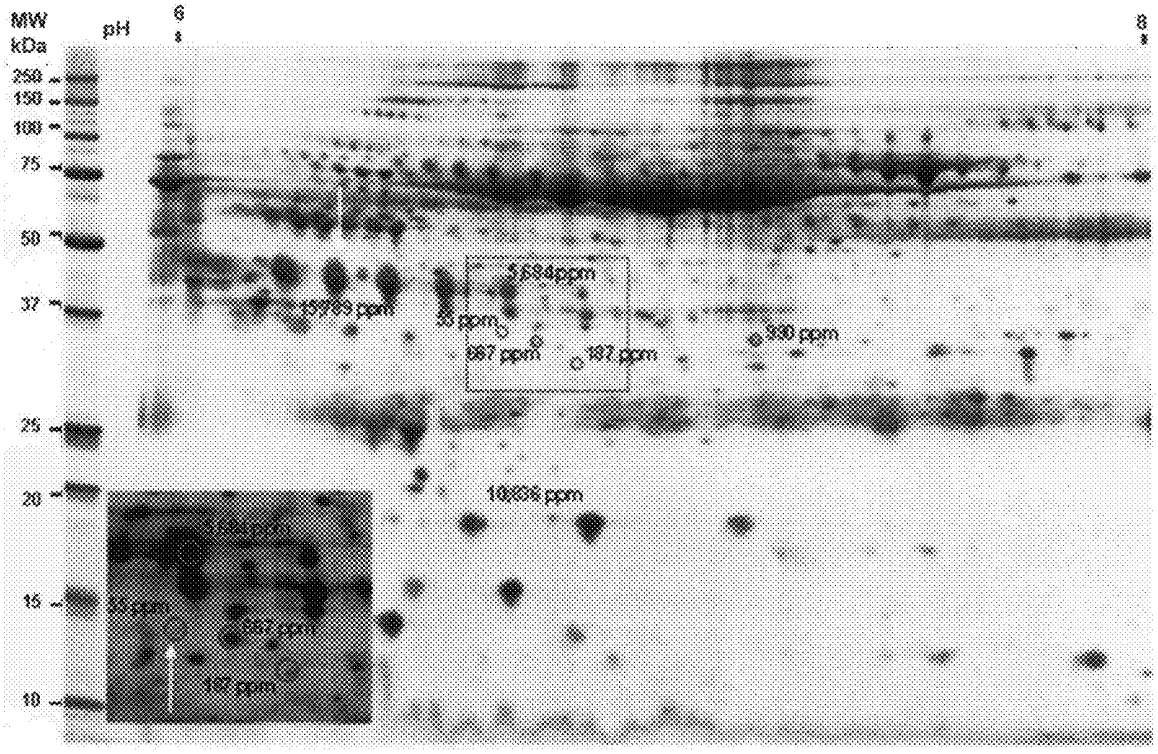
G01N 33/53	(2006.01)
G01N 33/561	(2006.01)

(52) **U.S. Cl.** **506/7**; 436/86; 435/29; 436/71; 435/7.92; 204/461

(57) **ABSTRACT**

The present invention relates to identification and uses of biomarkers for neurodegenerative disease, including Alzheimer's disease, and the related diseases. More specifically, the present invention relates to the identification of protein biomarkers useful for the screening, diagnosis, and differentiation of Alzheimer's disease from Parkinson's disease, other neurodegenerative diseases, and normal controls, and in the monitoring of Alzheimer's disease severity and disease mechanisms in patients.



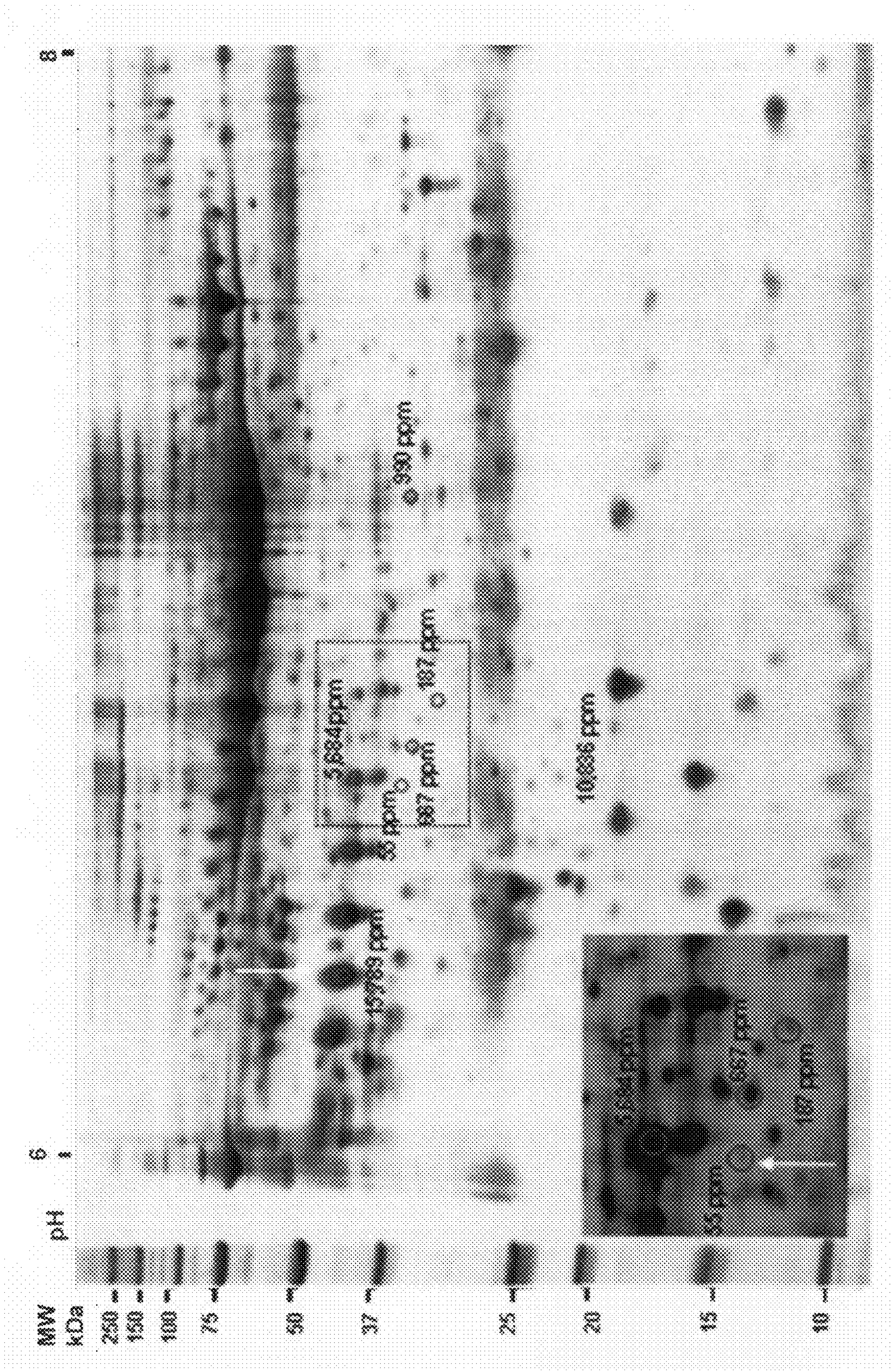

MW kDa
250
150
100
75
50
37
25
20
15
10
pH
6
8
157,769 ppm
5,684ppm
187 ppm
930 ppm
10,836 ppm

Fig. 1A

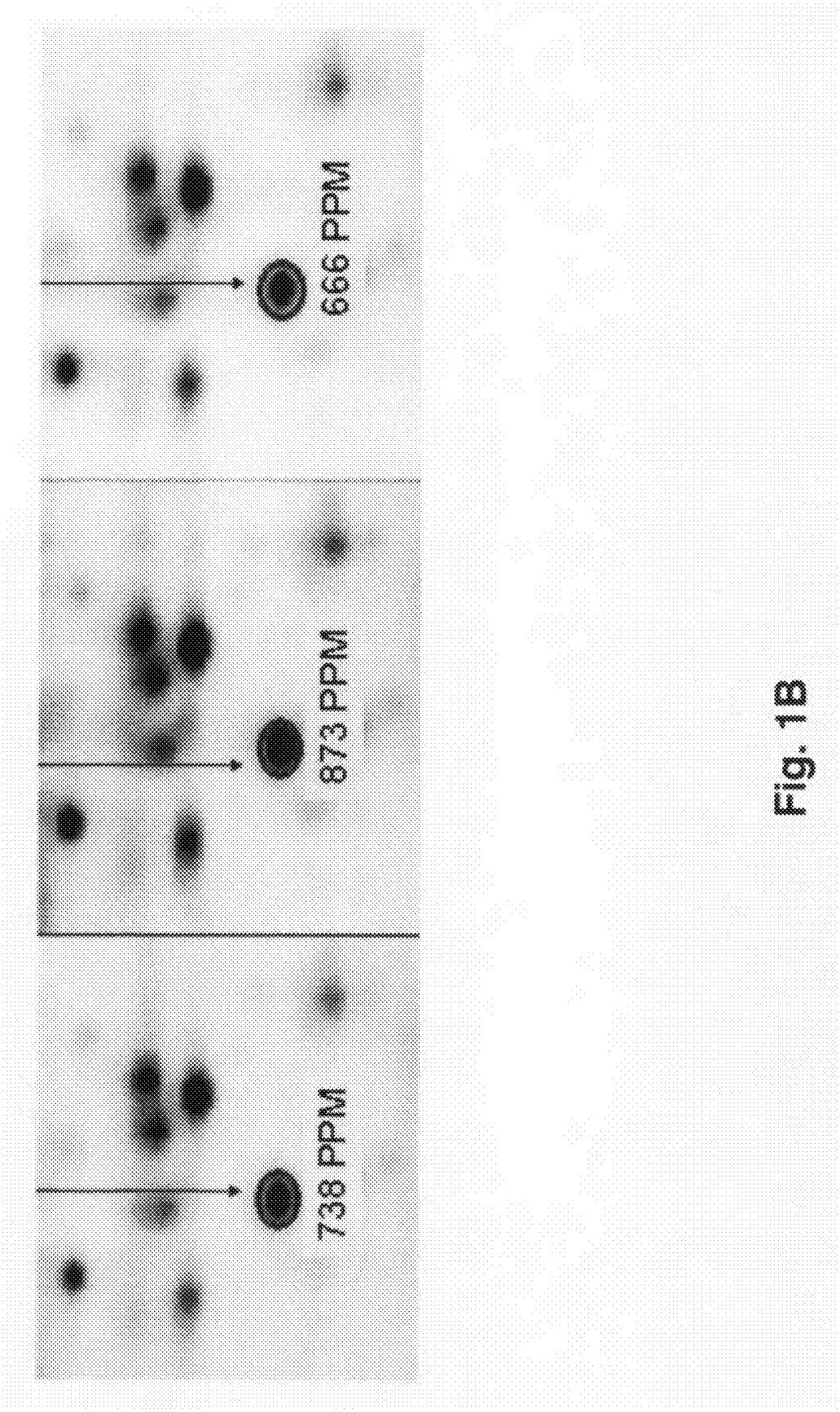


Fig. 1B

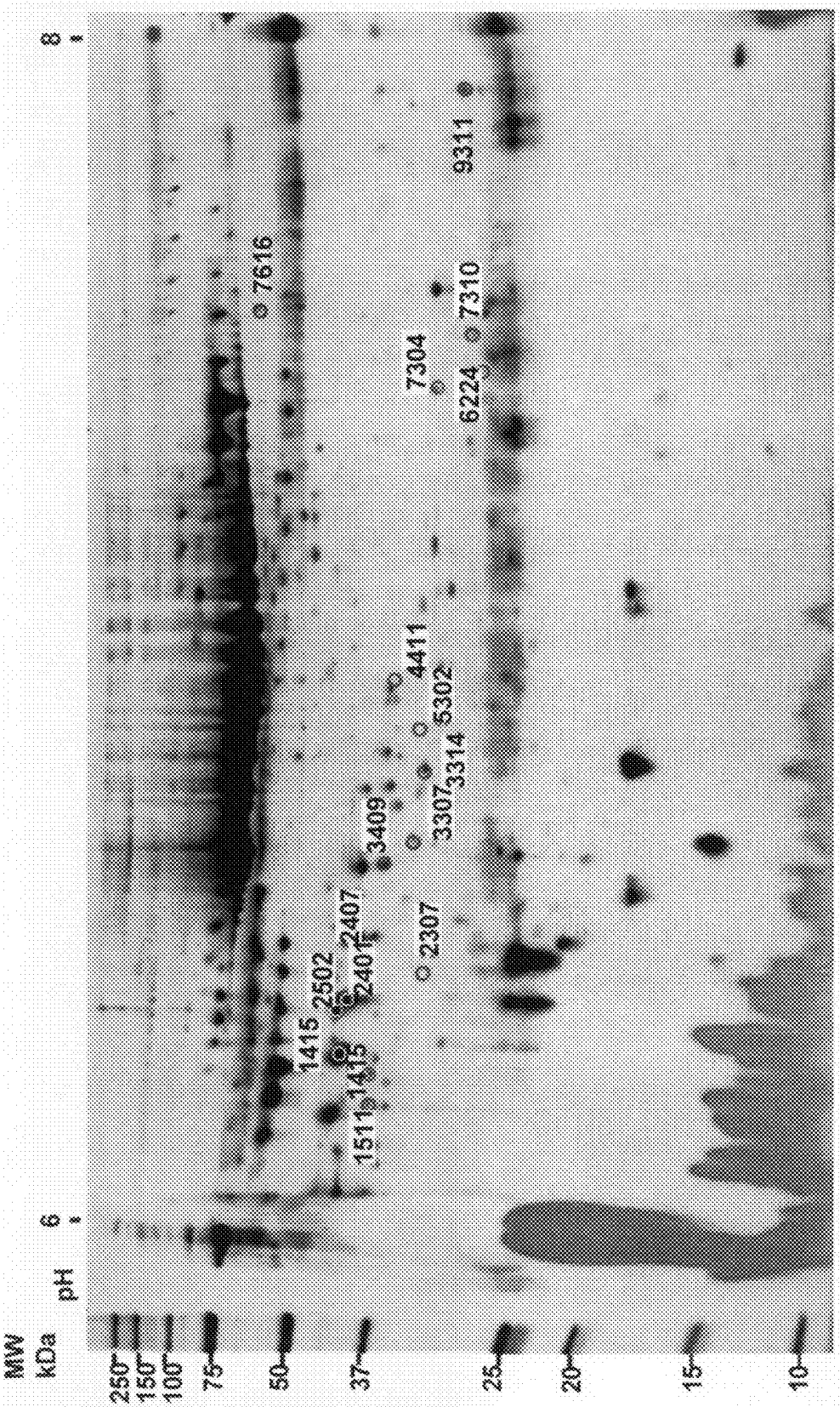

MW
kDa
pH
6
8
250
150
100
75
50
37
25
20
15
10
7616
9311
7310
7304
6224
4411
5302
3314
3307
3409
2407
2402
2502
1415
1511
2307

Fig. 2

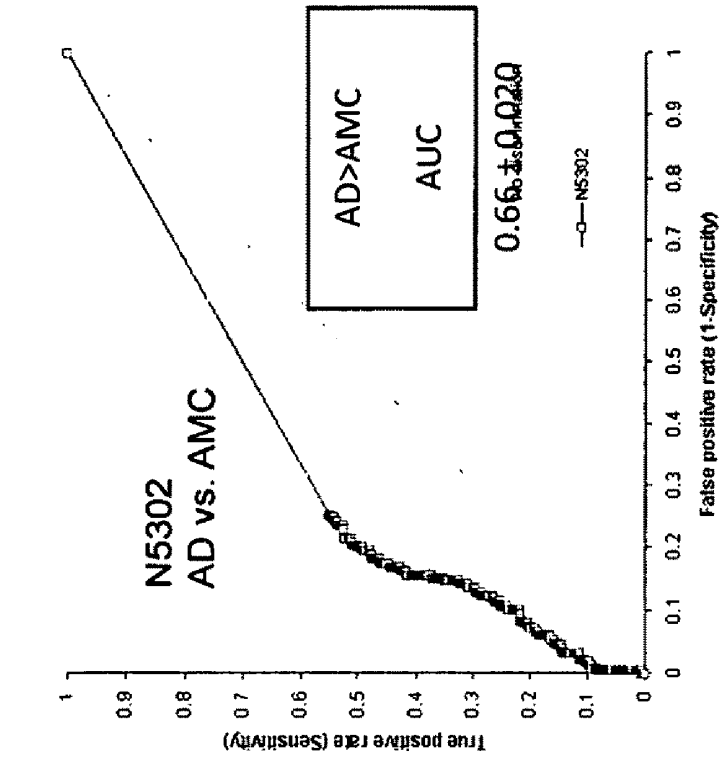

N5302
AD vs. AMC
AD>AMC
AUC
0.66±0.020
N5302
True positive rate (Sensitivity)
False positive rate (1-Specificity)

Fig. 3B

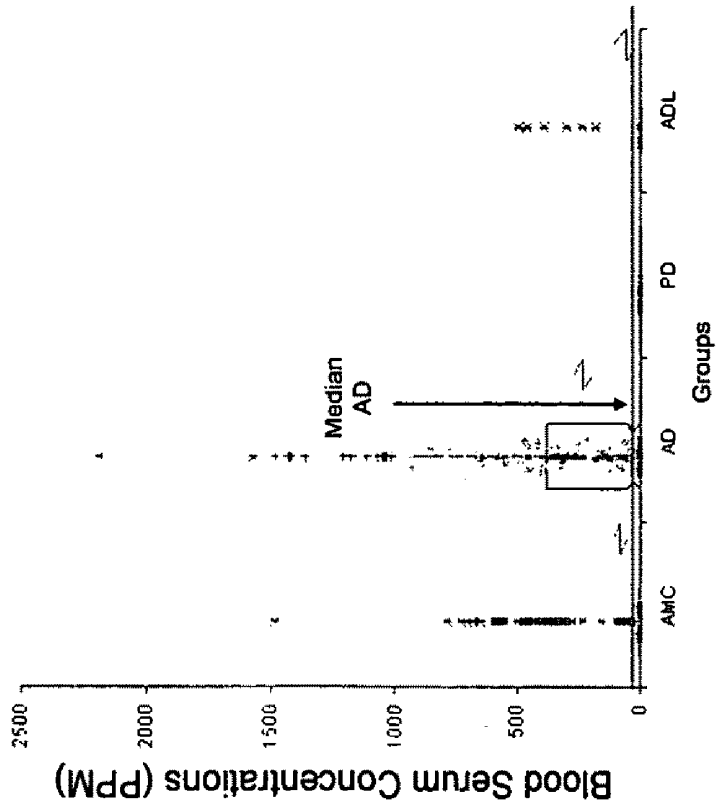

Blood Serum Concentrations (PPM)
Median AD
AMC
AD
PD
ADL
Groups

Fig. 3A

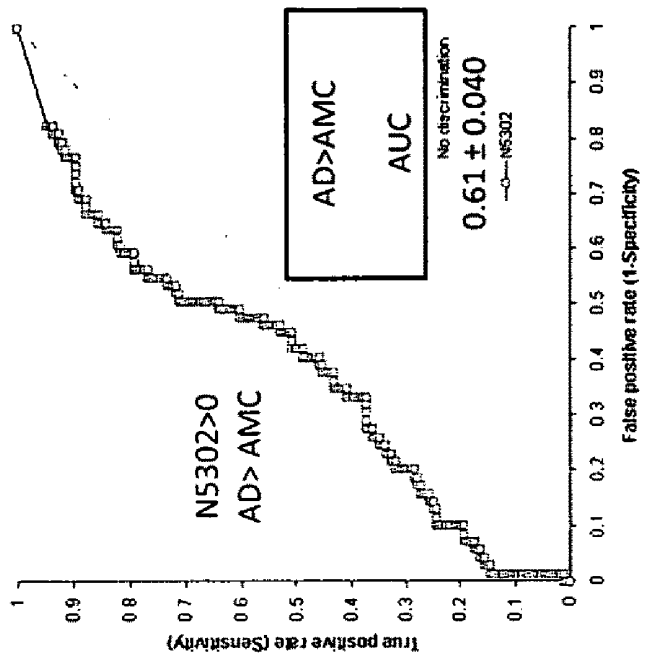

AD>AMC
AUC
0.61 ± 0.040
N5302>0
AD> AMC
True positive rate (Sensitivity)
False positive rate (1-Specificity)

Fig. 4B

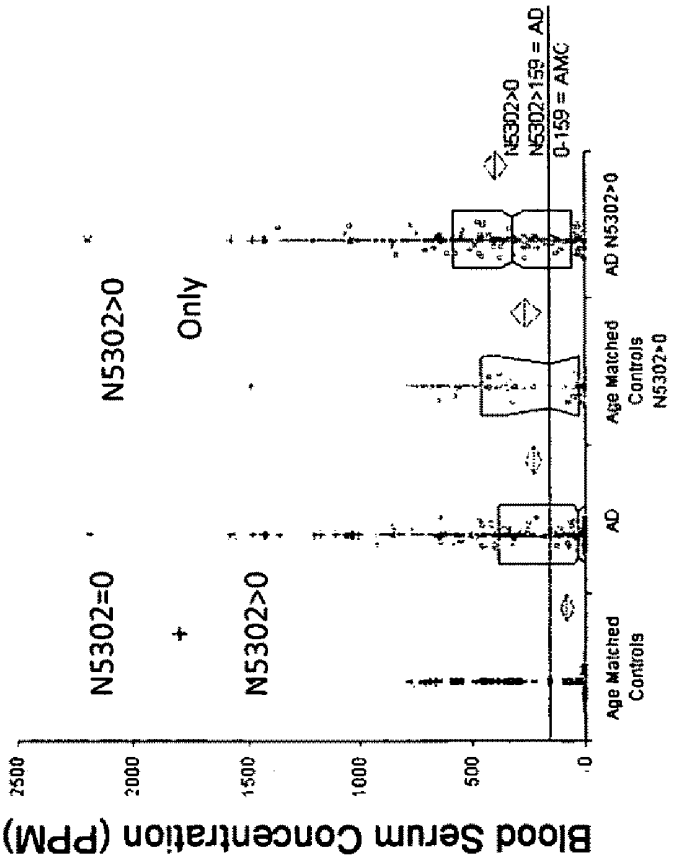

Blood Serum Concentration (PPM)
N5302=0
+
N5302>0
Only
N5302>0
N5302>159 = AD
0-159 = AMC
Age Matched Controls
AD
AD N5302>0
Age Matched Controls N5302>0

Fig. 4A

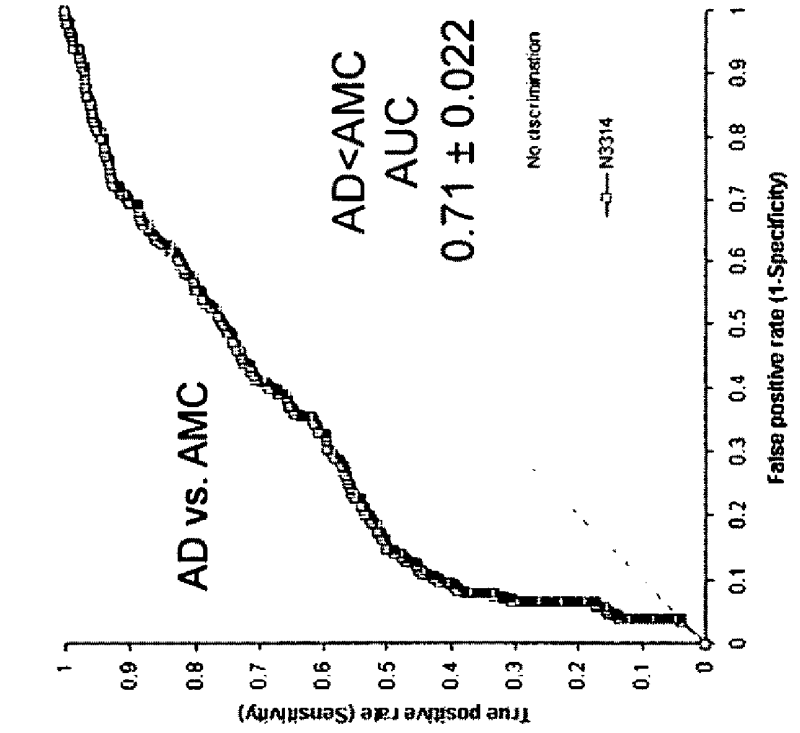

AD vs. AMC
AD<AMC
AUC
0.71 ± 0.022
No discrimination
N3314
True positive rate (Sensitivity)
False positive rate (1-Specificity)

Fig. 5B

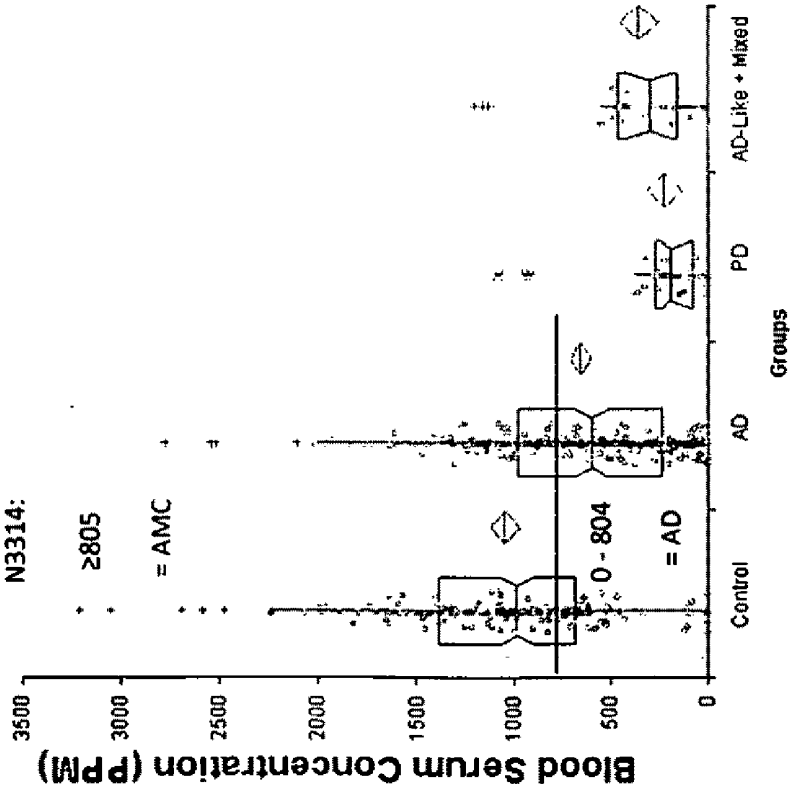

Blood Serum Concentration (PPM)
N3314:
≥805
= AMC
0 - 804
= AD
Control
AD
PD
AD-Like + Mixed
Groups

Fig. 5A

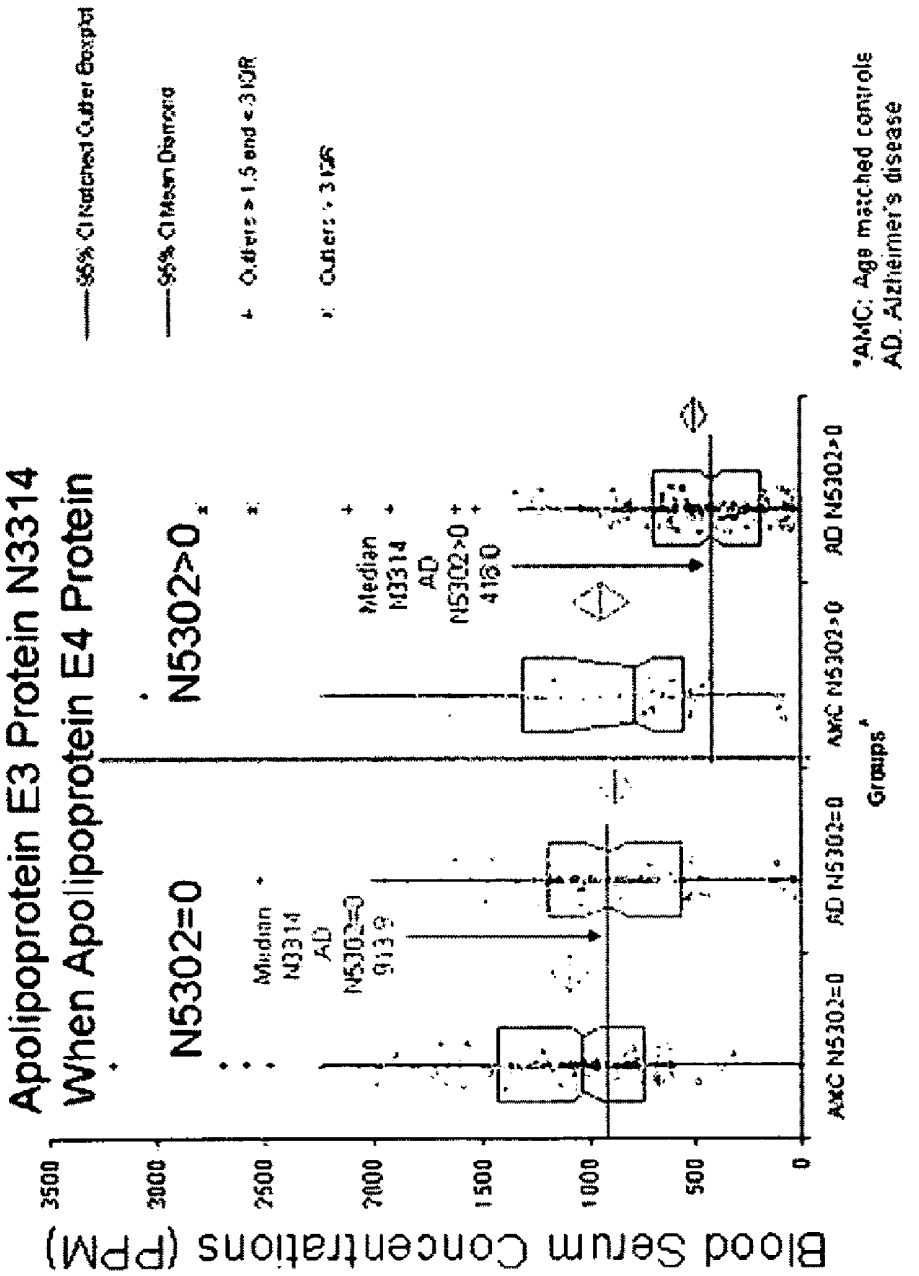

Apolipoprotein E3 Protein N3314
When Apolipoprotein E4 Protein
N5302=0
N5302>0
Median
N3314
AD
N5302=0
919.9
Median
N3314
AD
N5302>0
4160
Blood Serum Concentrations (PPM)
3500
3000
2500
2000
1500
1000
500
0
AMC N5302=0
AD N5302=0
AMC N5302>0
AD N5302>0
Groups
*AMC: Age matched controls
AD: Alzheimer's disease

Fig. 6

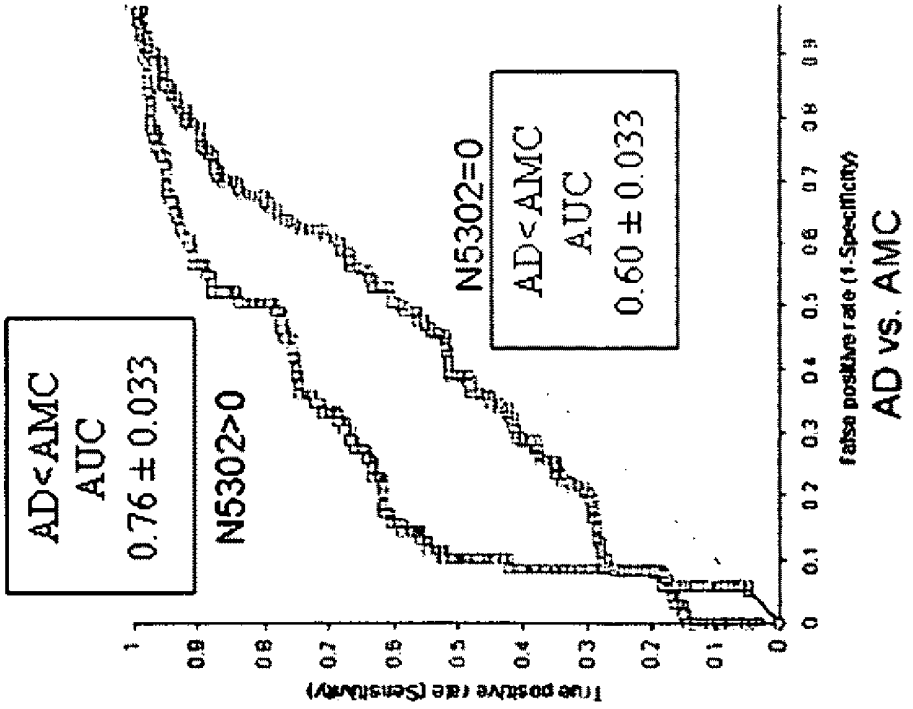

AD<AMC
AUC
0.76 ± 0.033
N5302>0
N5302=0
AD<AMC
AUC
0.60 ± 0.033
True positive rate (Sensitivity)
False positive rate (1-Specificity)
AD vs. AMC

Fig. 7A

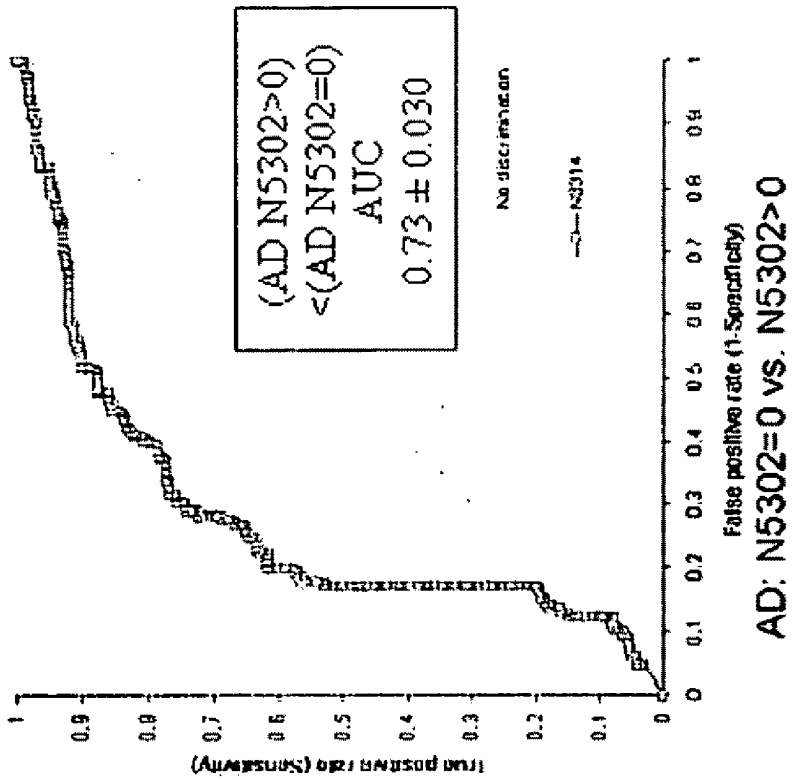

(AD N5302>0)
<(AD N5302=0)
AUC
0.73 ± 0.030
No discrimination
N5314
True positive rate (Sensitivity)
False positive rate (1-Specificity)
AD: N5302=0 vs. N5302>0

Fig. 7B

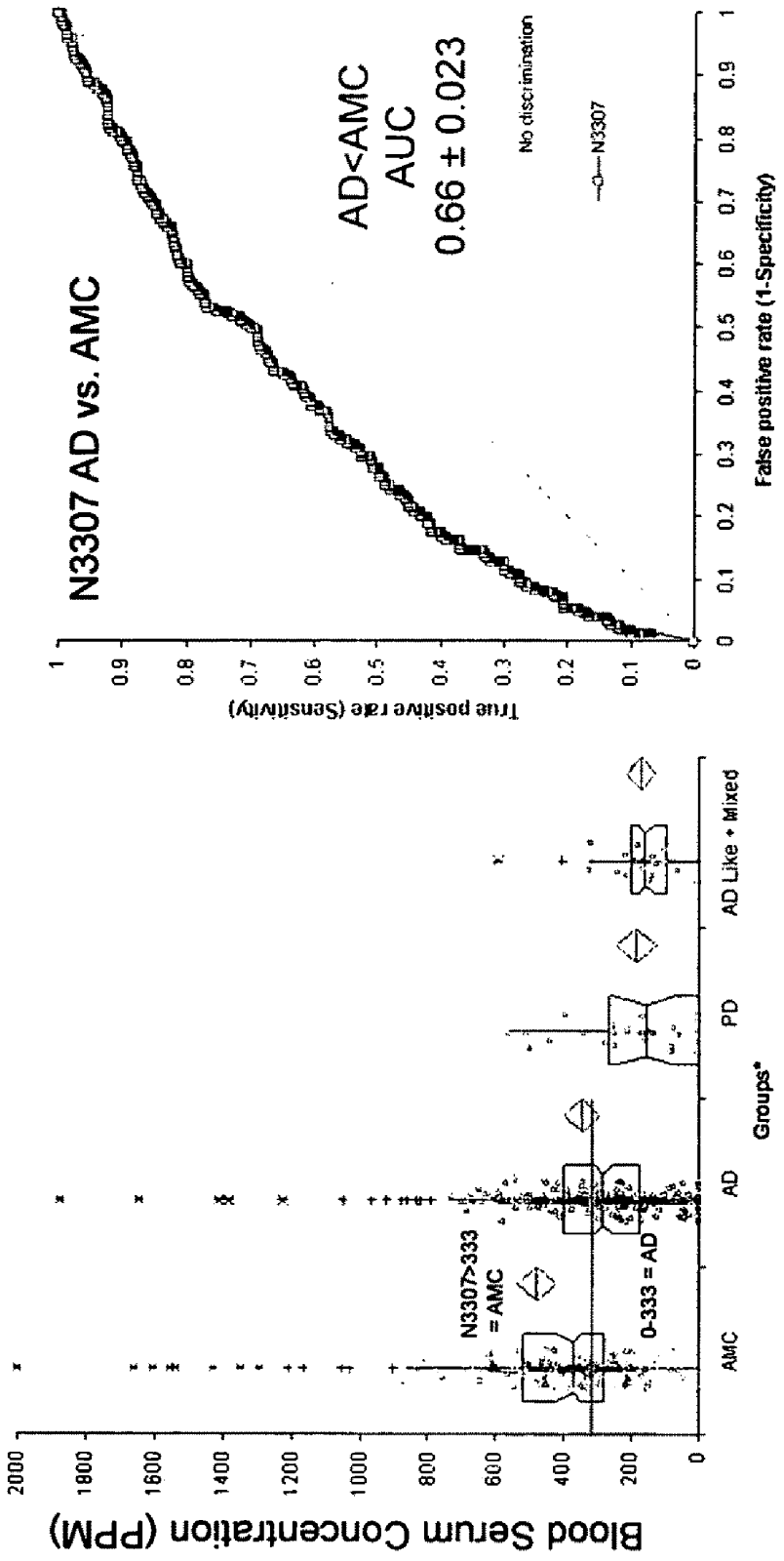

Blood Serum Concentration (PPM)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
AMC
AD
PD
AD Like + Mixed
Groups*
N3307>333 = AMC
0-333 = AD

Fig. 8A

N3307 AD vs. AMC
AD<AMC
AUC
0.66 ± 0.023
No discrimination
N3307
True positive rate (Sensitivity)
False positive rate (1-Specificity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

Fig. 8B

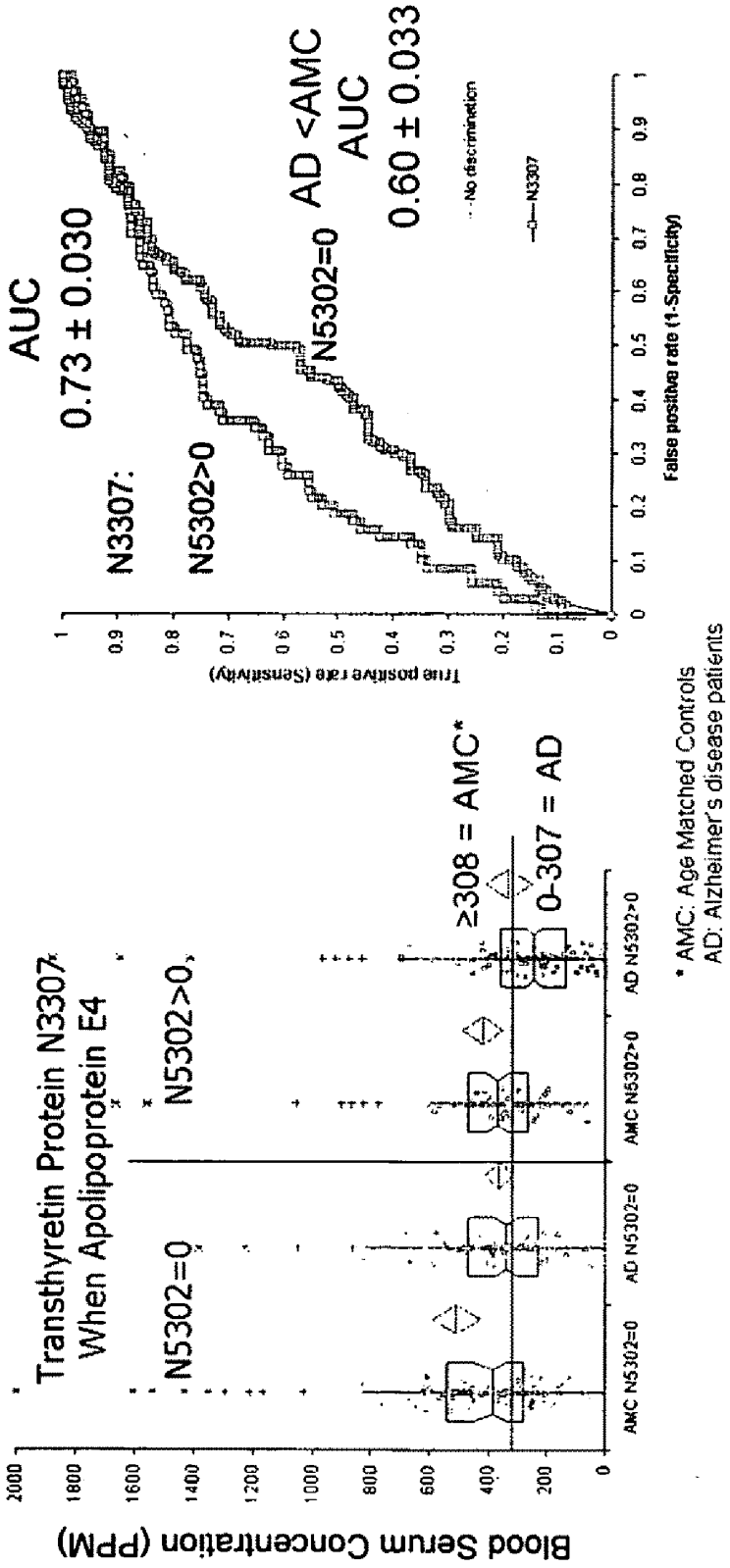

Transthyretin Protein N3307
When Apolipoprotein E4
N5302=0
N5302>0,
≥308 = AMC*
0-307 = AD
Blood Serum Concentration (PPM)
AMC N5302=0
AD N5302=0
AMC N5302>0
AD N5302>0
* AMC: Age Matched Controls
AD: Alzheimer's disease patients
AD <AMC
AUC
0.73 ± 0.030
N3307:
N5302>0
N5302=0
AD <AMC
AUC
0.60 ± 0.033
True positive rate (Sensitivity)
False positive rate (1-Specificity)
No discrimination
N3307

Fig. 9A

Fig. 9B

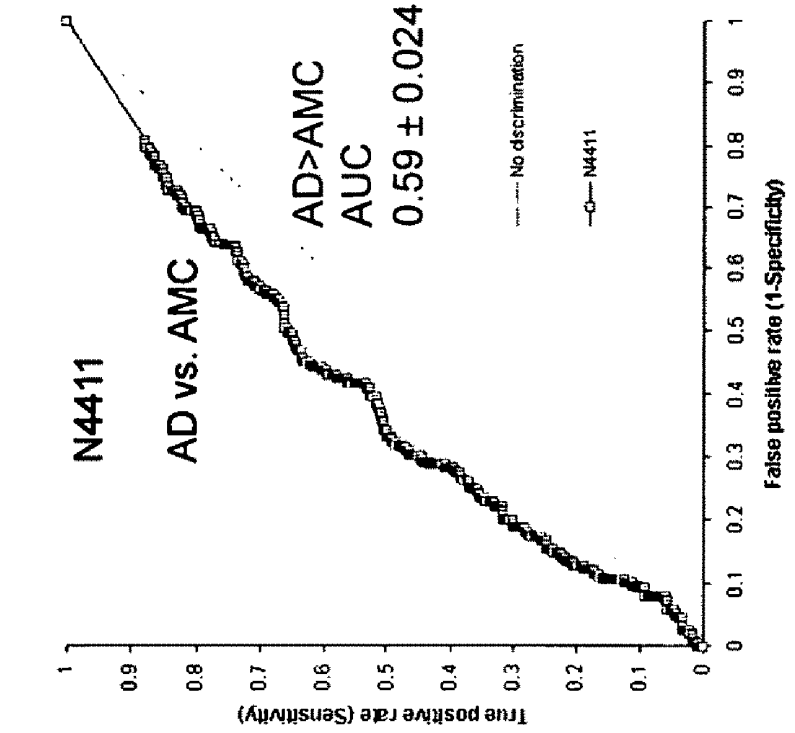


Fig. 10B

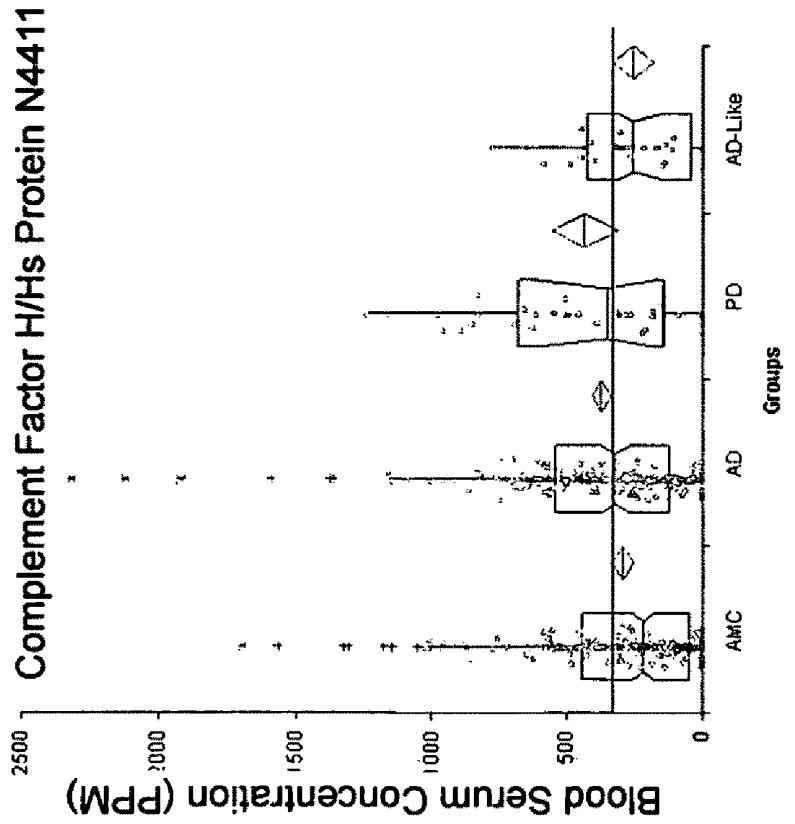


Fig. 10A

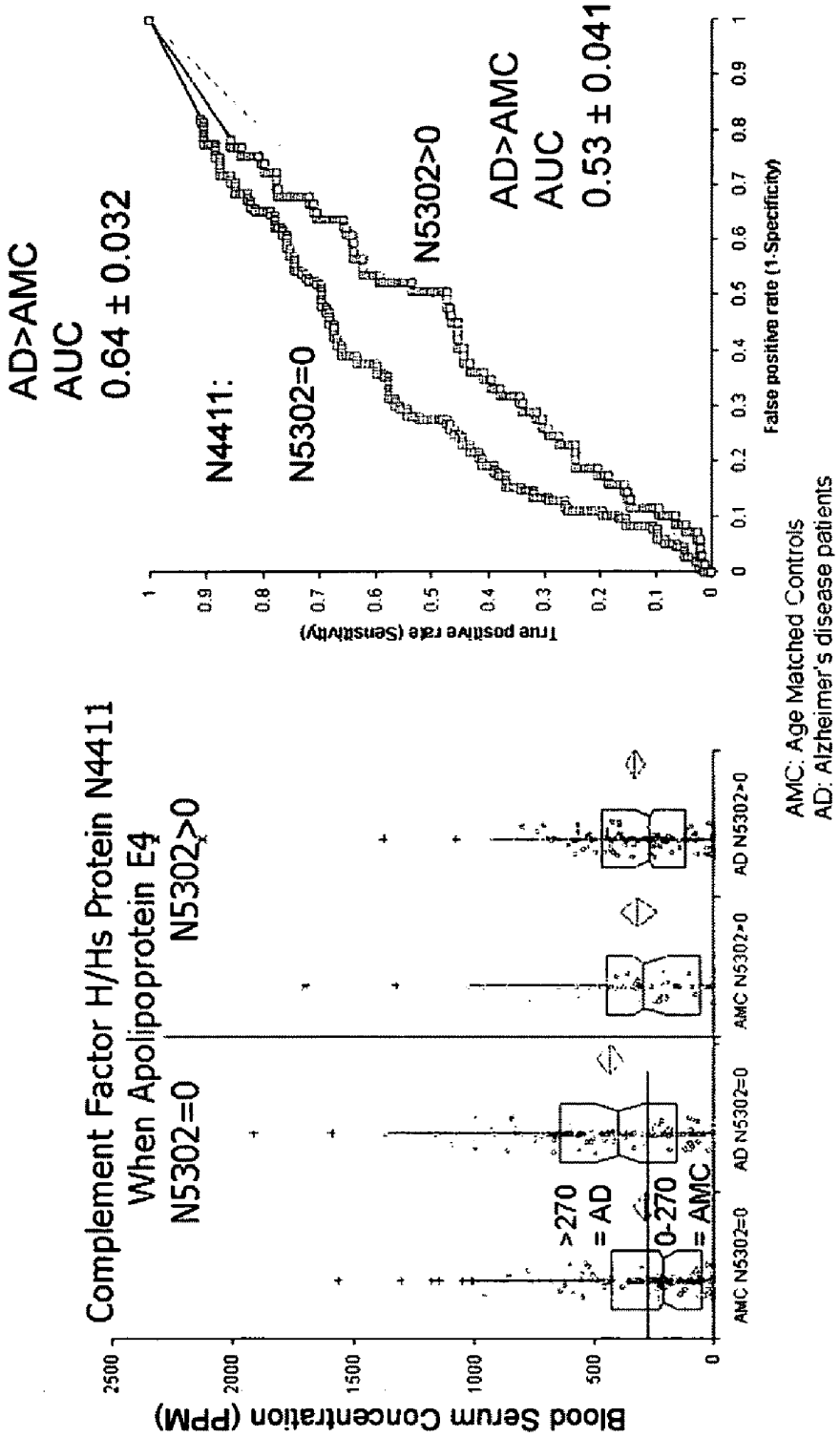


Fig. 11A

Fig. 11B

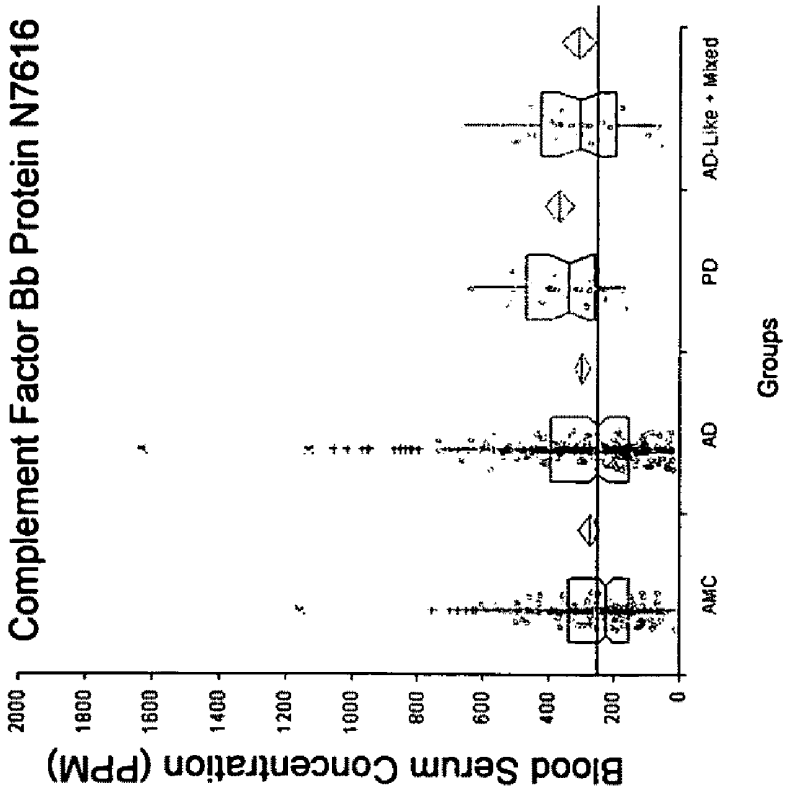


Fig. 12A

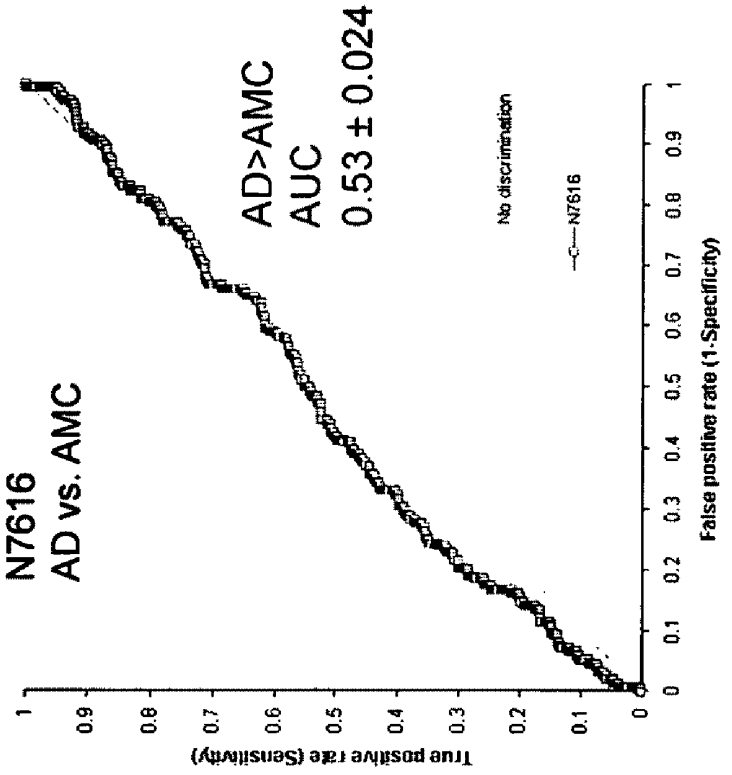


Fig. 12B

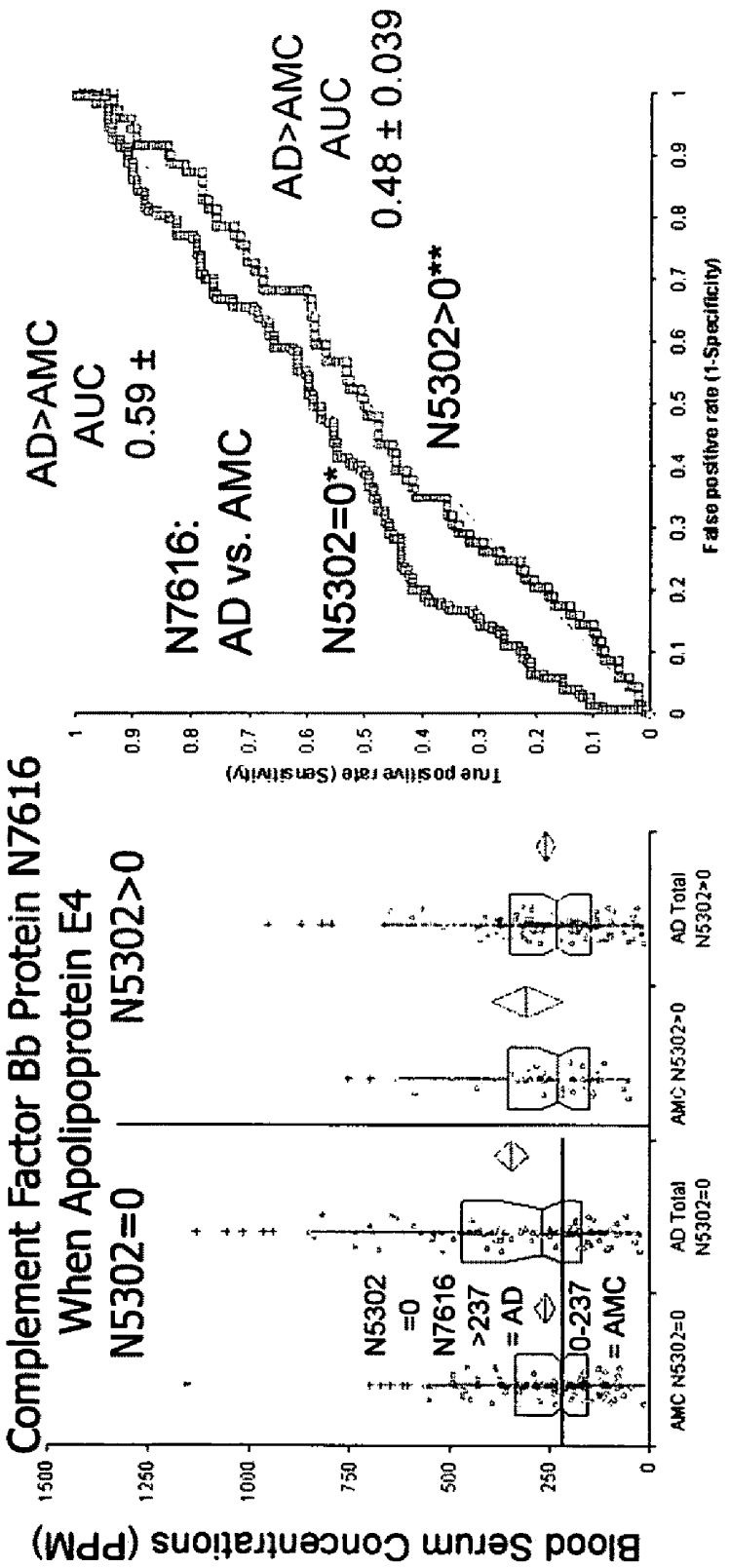


Fig. 13A

Fig. 13B

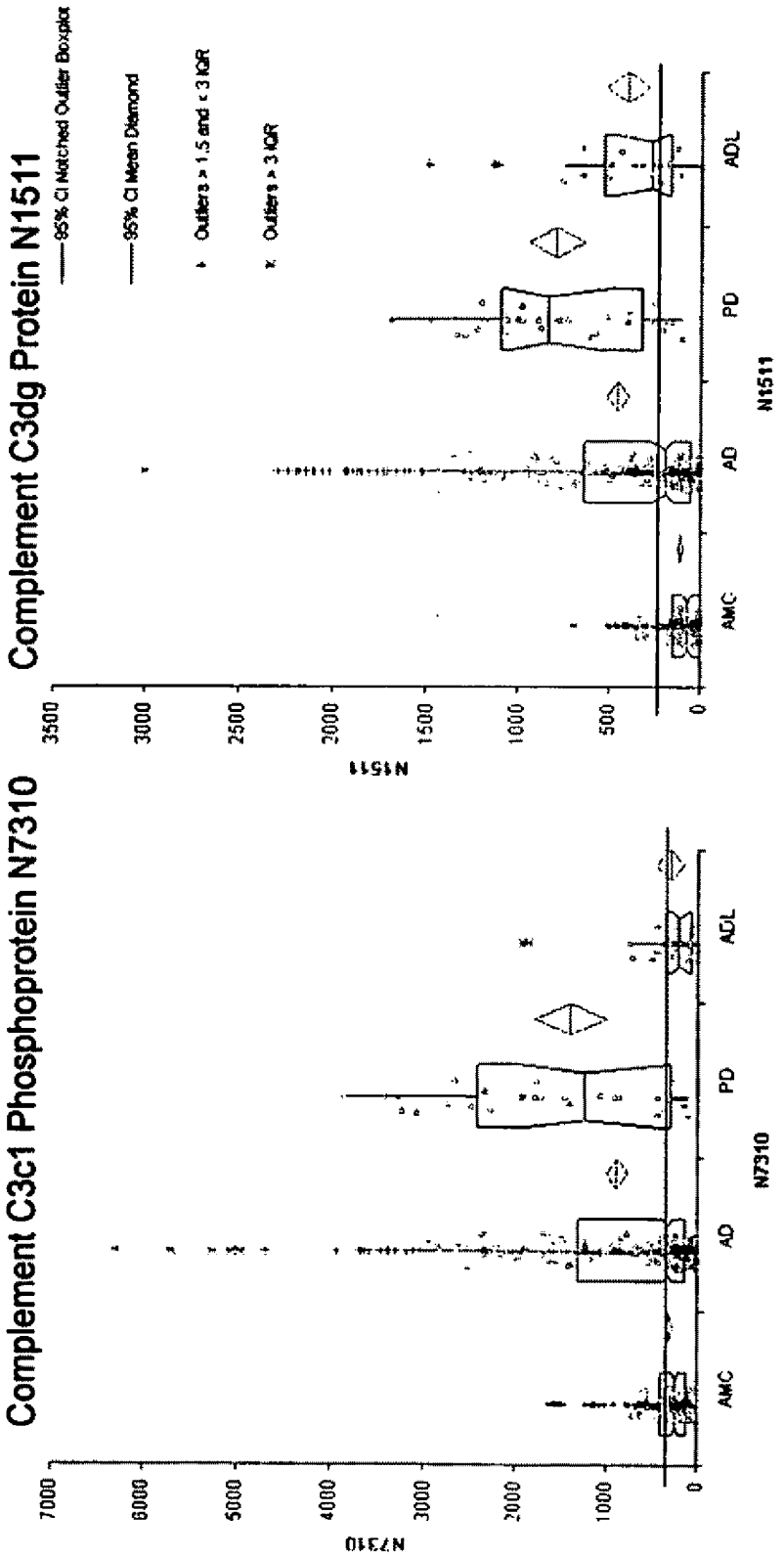


Fig. 14A

Fig. 14B

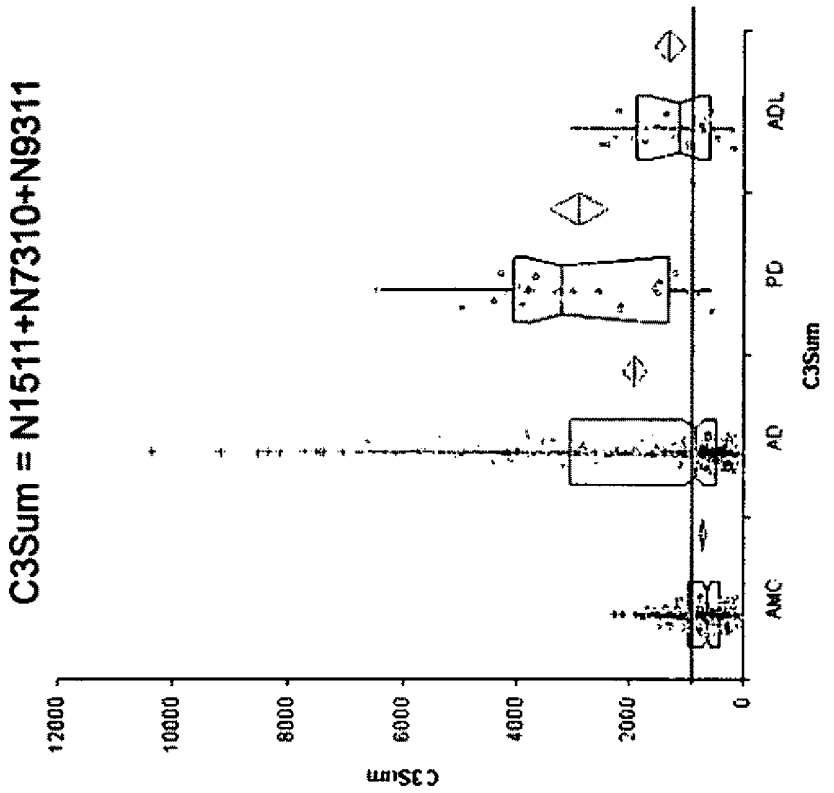


Fig. 14D

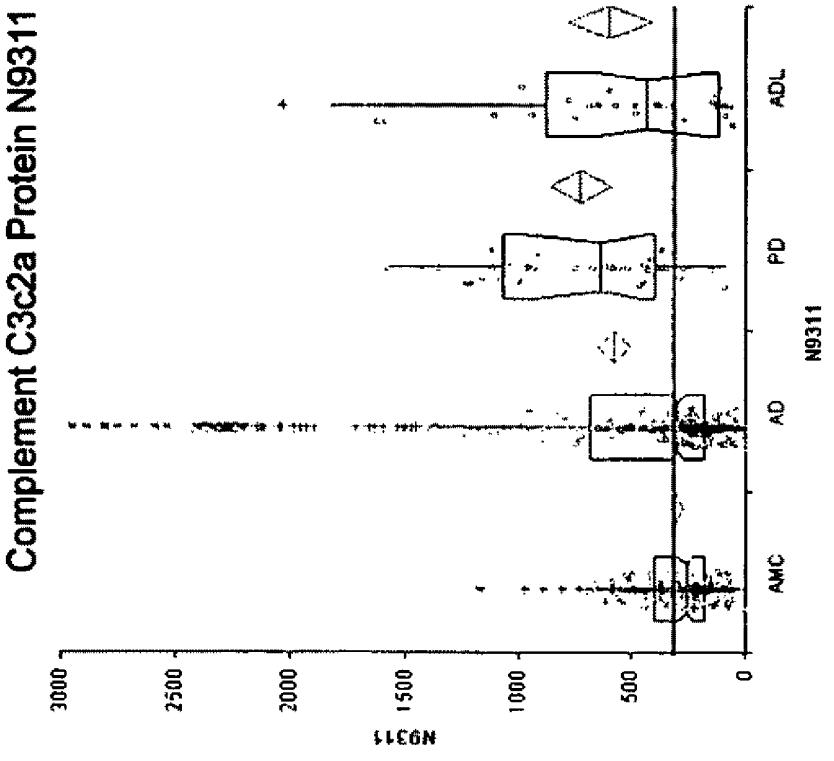


Fig. 14C

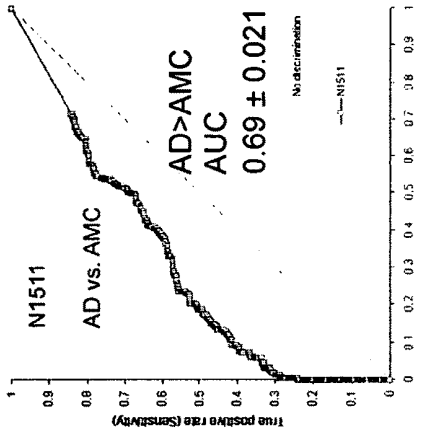


Fig. 15A

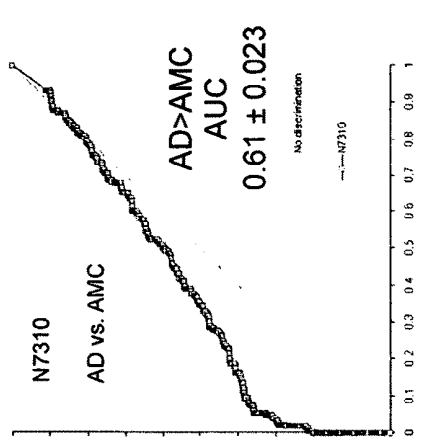


Fig. 15B

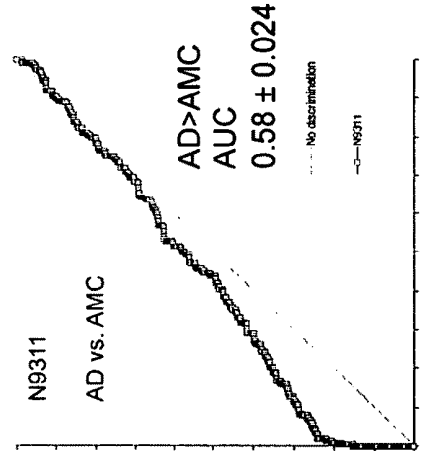


Fig.15D

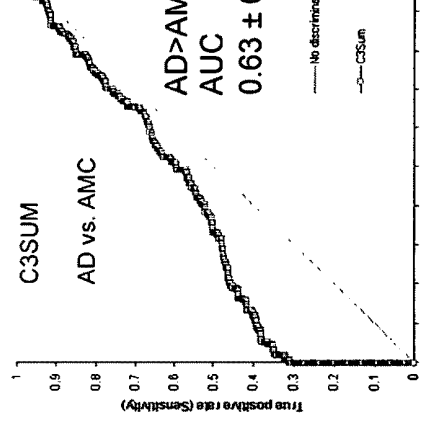


Fig. 15C

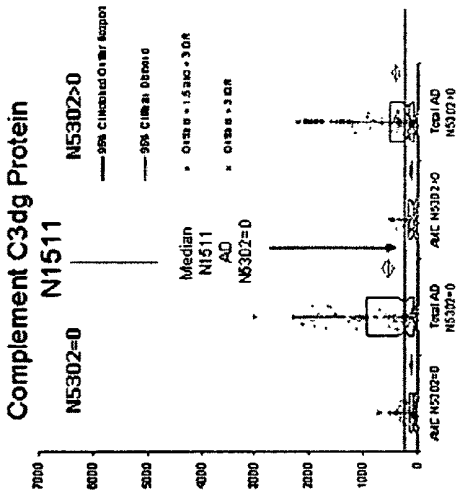


Fig. 16A

Fig. 16B

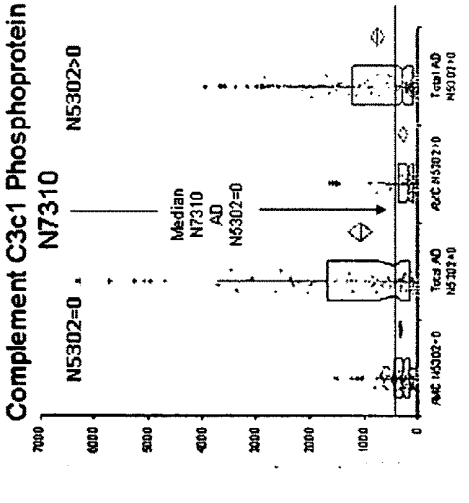


Fig. 16C

Fig. 16D

Blood Serum Concentration (PPM)

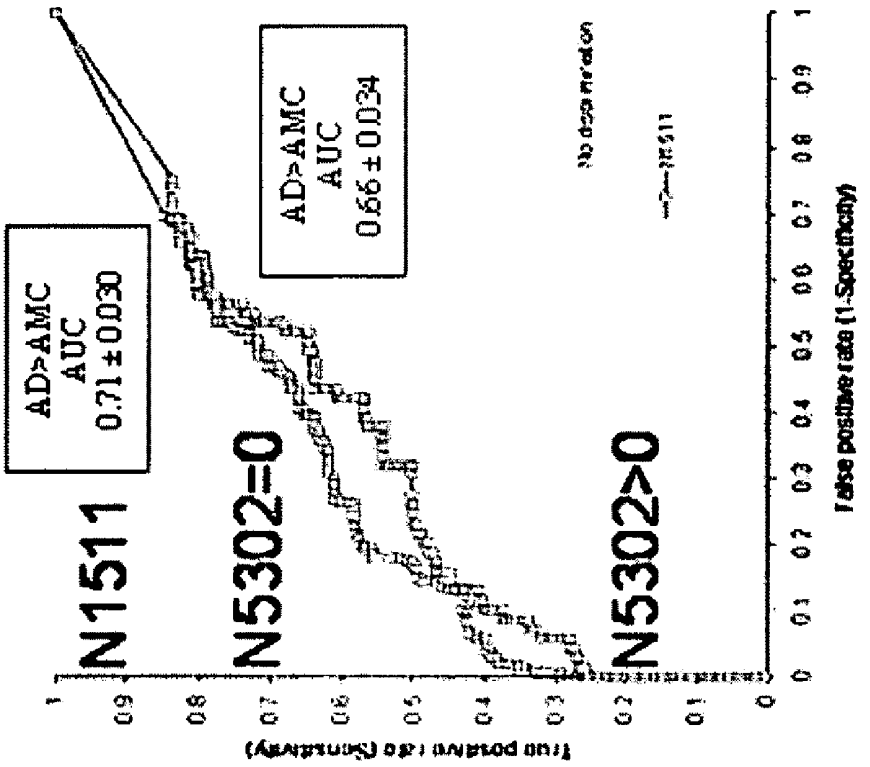


Fig. 17B

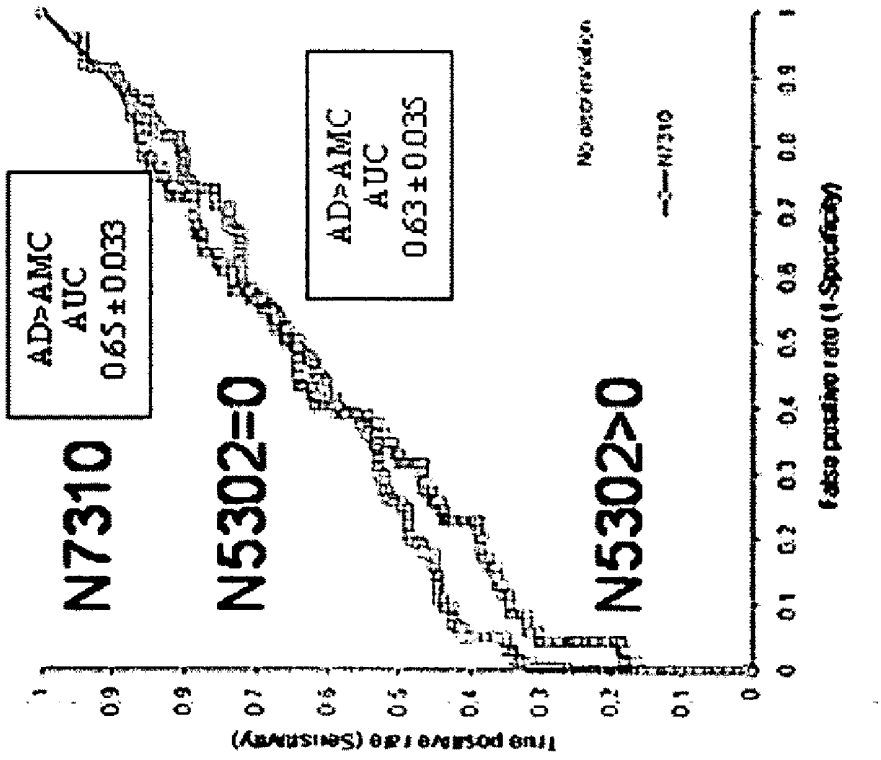


Fig. 17A

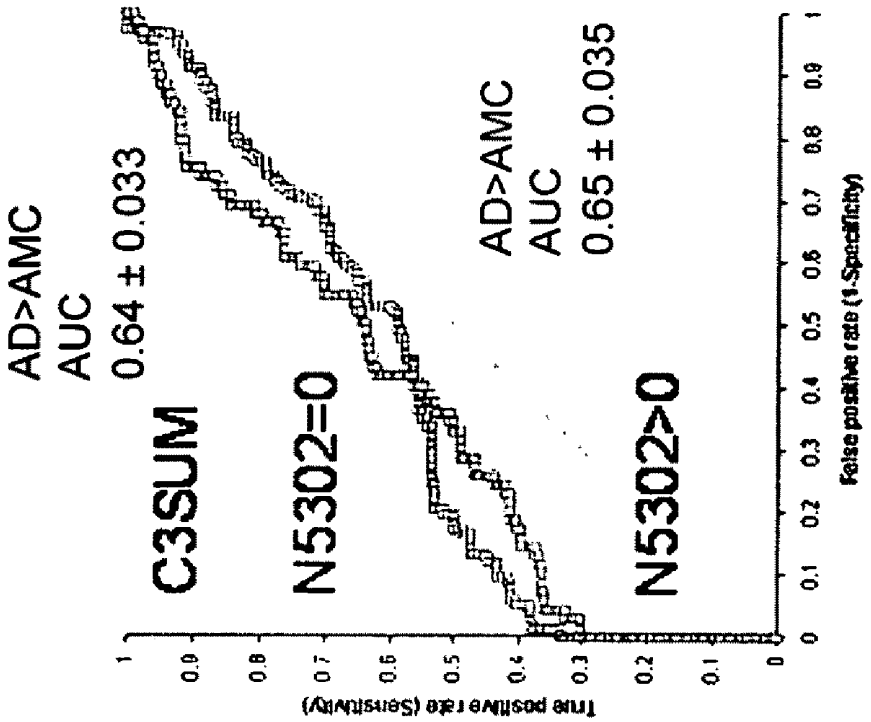


Fig. 17D

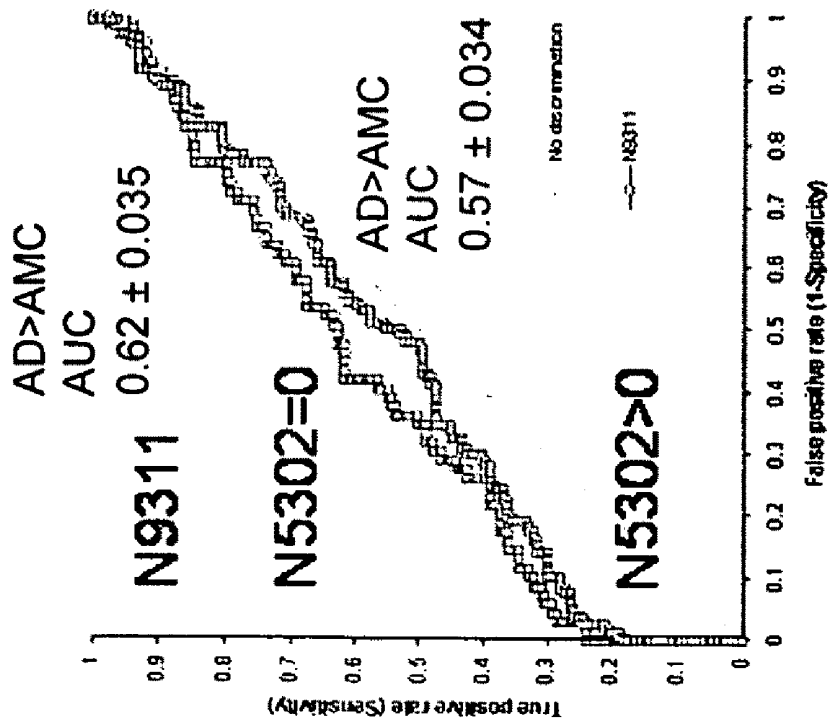


Fig. 17C

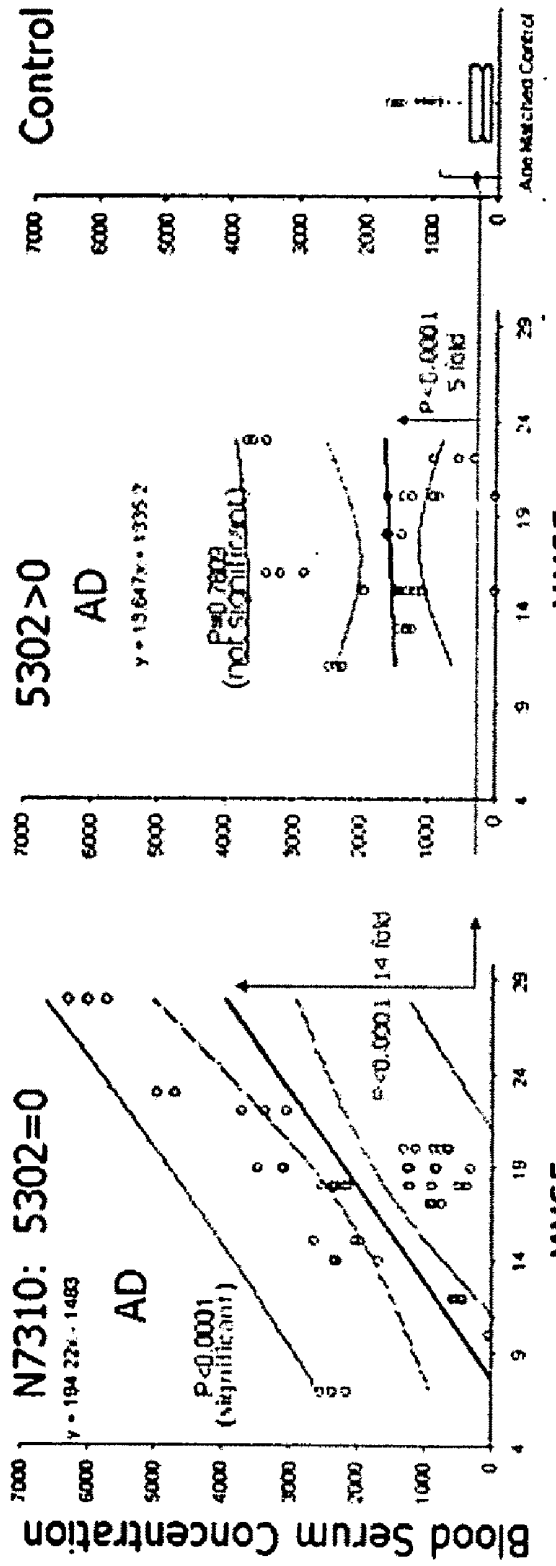


Fig. 18A

Fig. 18B

Fig. 18C

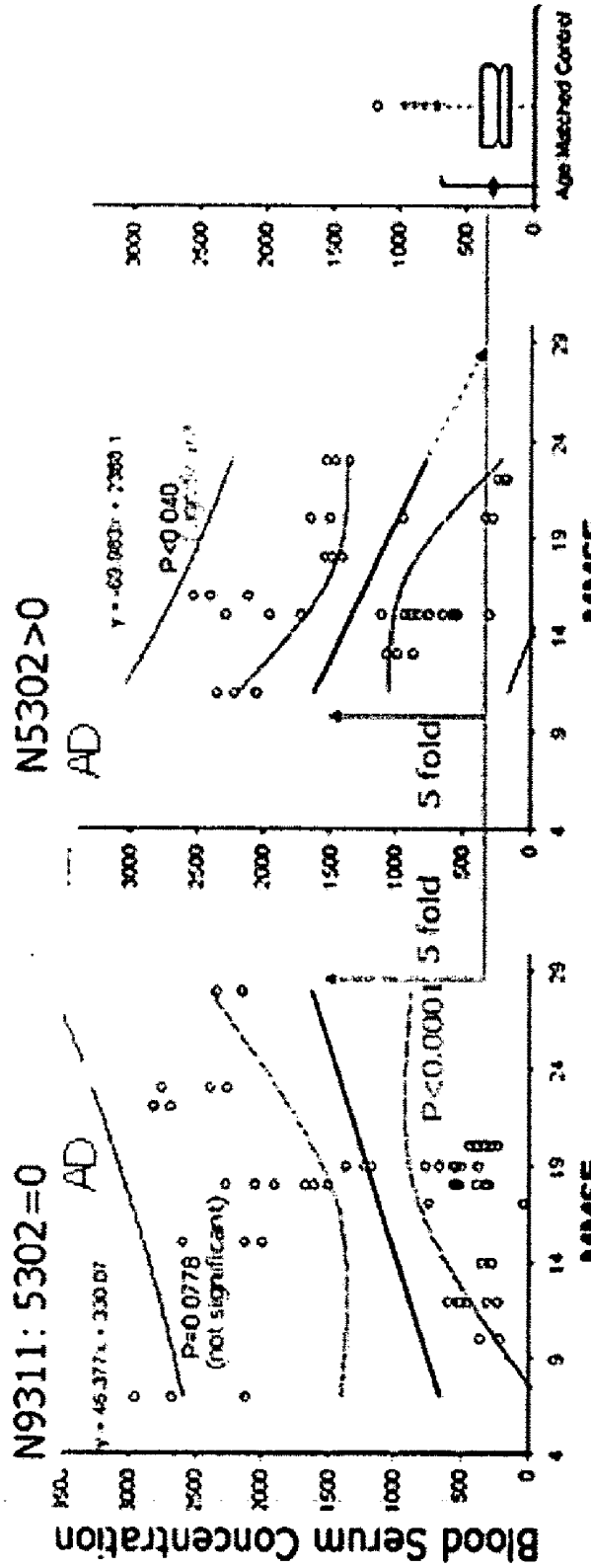


Fig. 18D

Fig. 18E

Fig. 18F

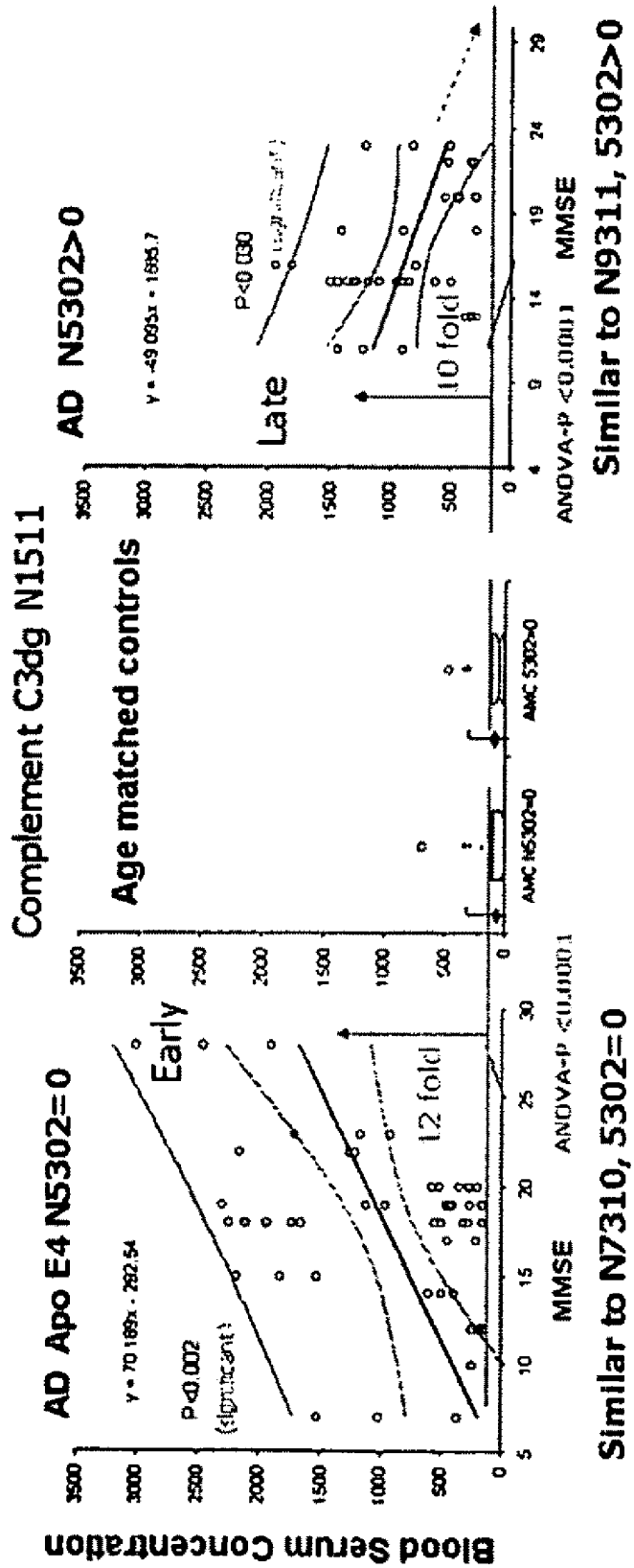


Fig. 19A

Fig. 19B

Fig. 19C

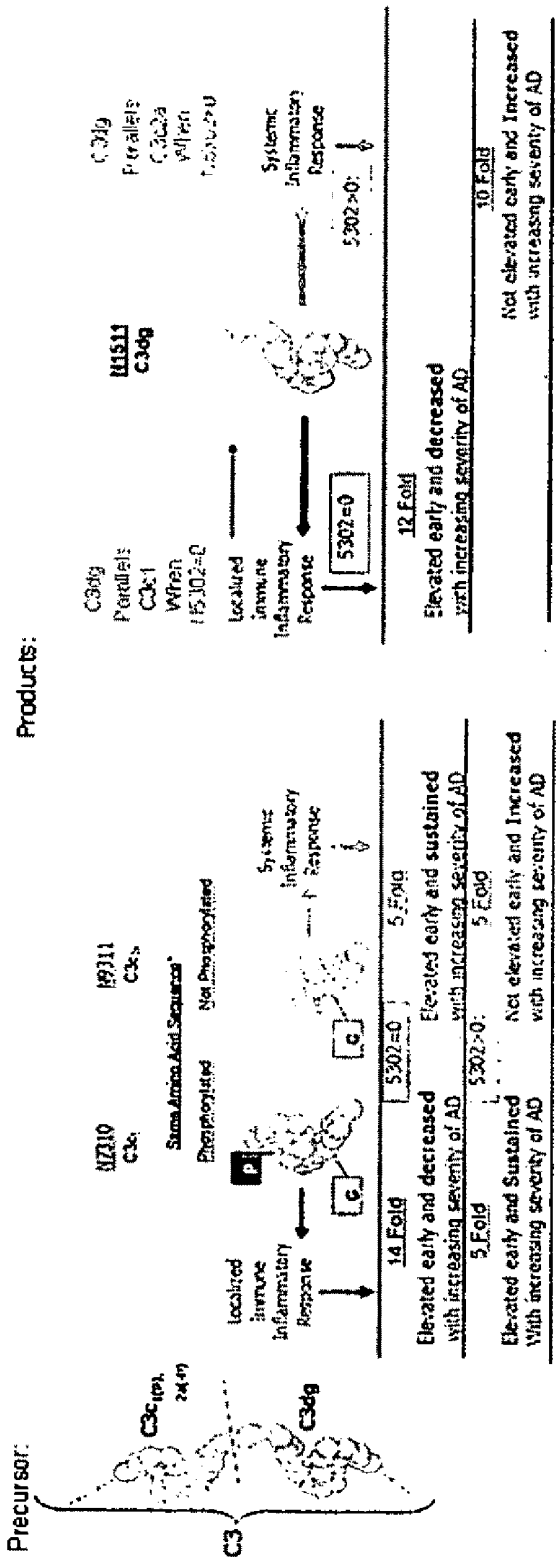

Precursor:
C3
Products:
NZ310
M9311
Same Amino Acid Sequence*
Phosphorylated
Not Phosphorylated
Localized Immune Inflammatory Response
Systemic Inflammatory Response
14 Fold
5 Fold
Elevated early and decreased with increasing severity of AD
Elevated early and sustained with increasing severity of AD
Elevated early and Sustained With increasing severity of AD
Not elevated early and Increased with increasing severity of AD
H1511
C3dg
12 Fold
10 Fold

Fig. 20

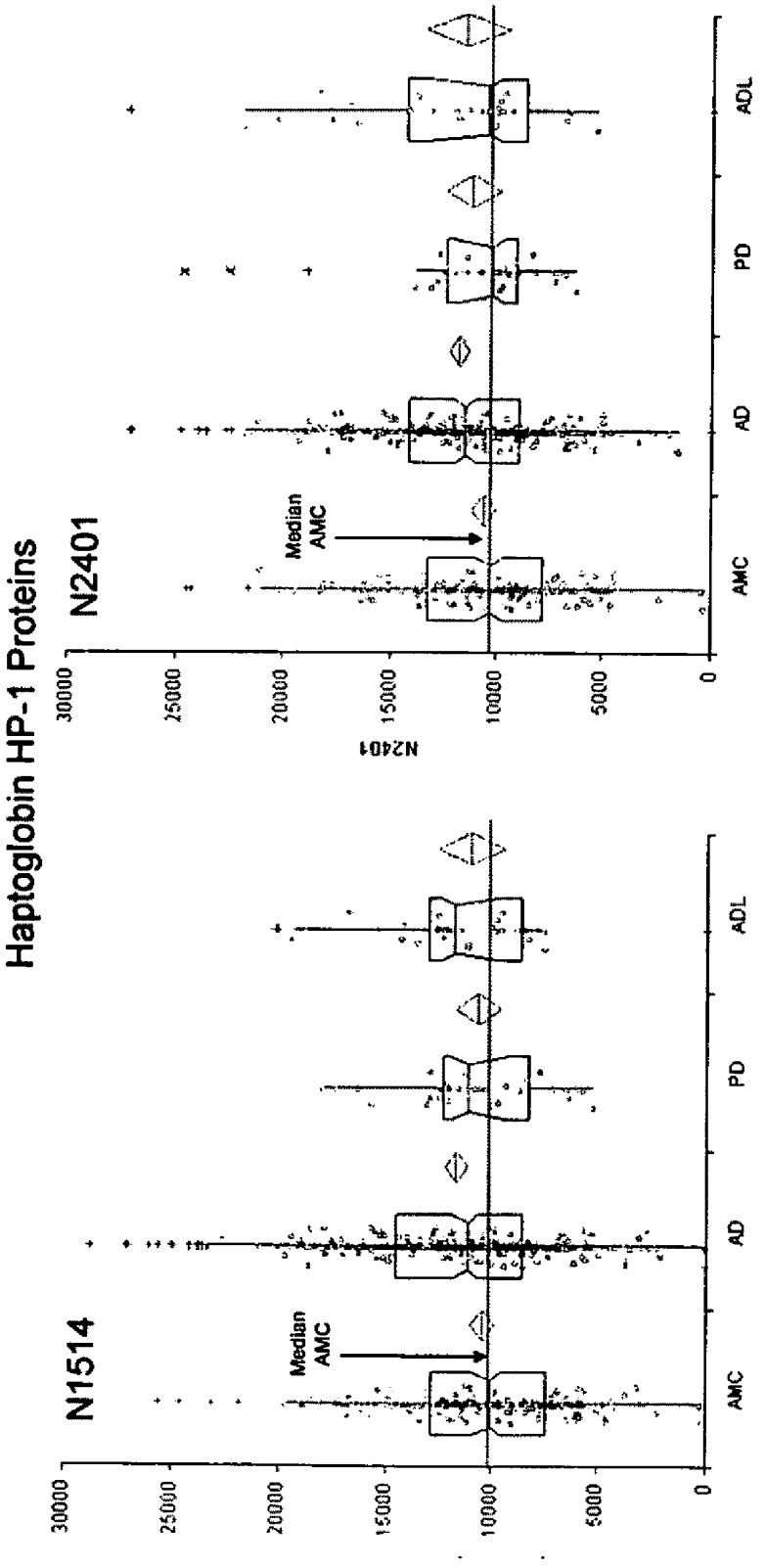


Fig. 21B

Fig. 21A

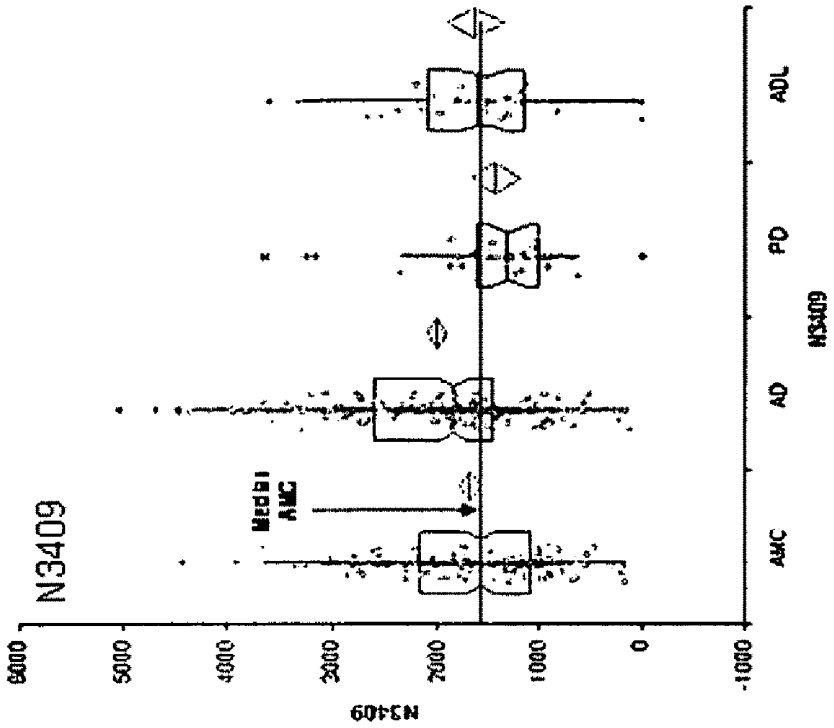


Fig. 21D

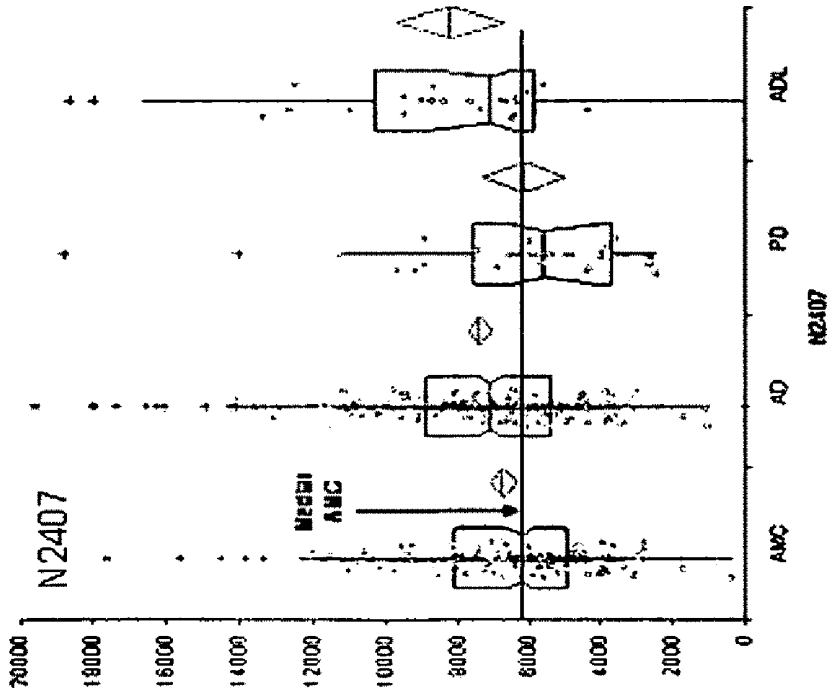


Fig. 21C

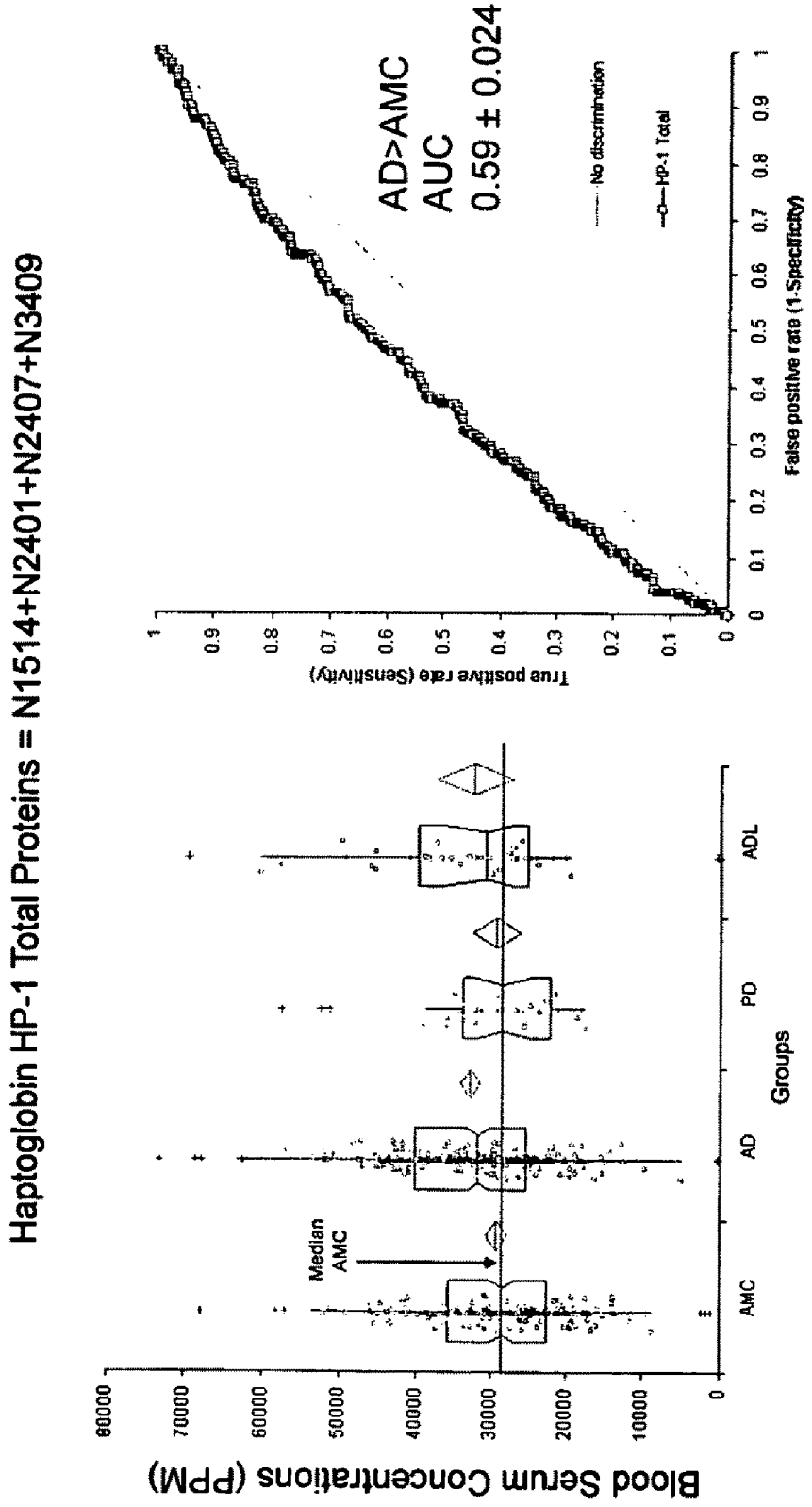


Fig. 22A

Fig. 22B

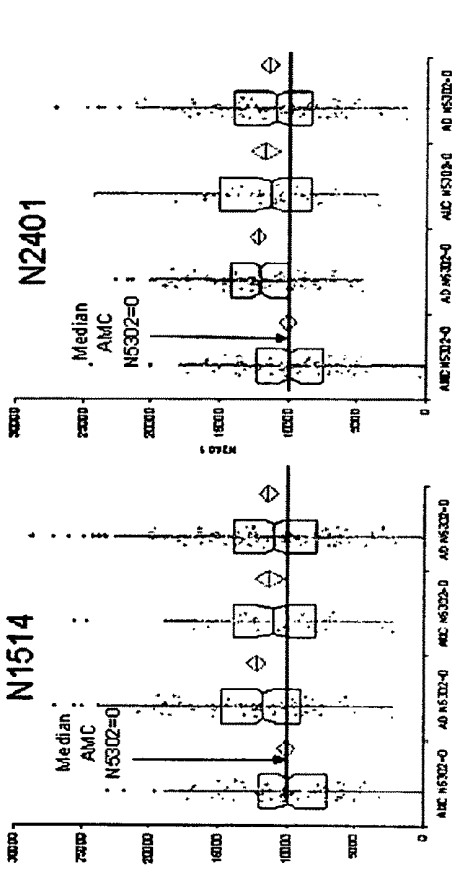


Fig. 23A

Fig. 23B

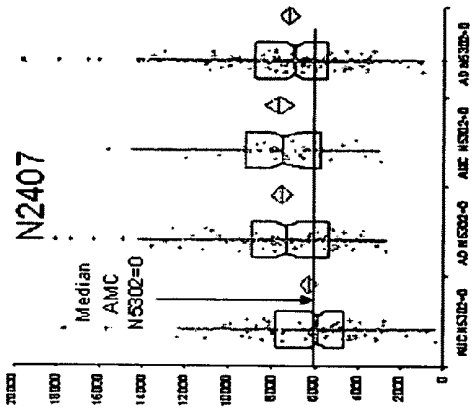


Fig. 23C

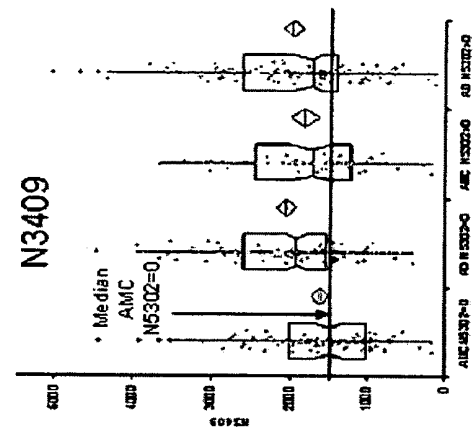


Fig. 23D

Blood Serum Concentrations (PPM)

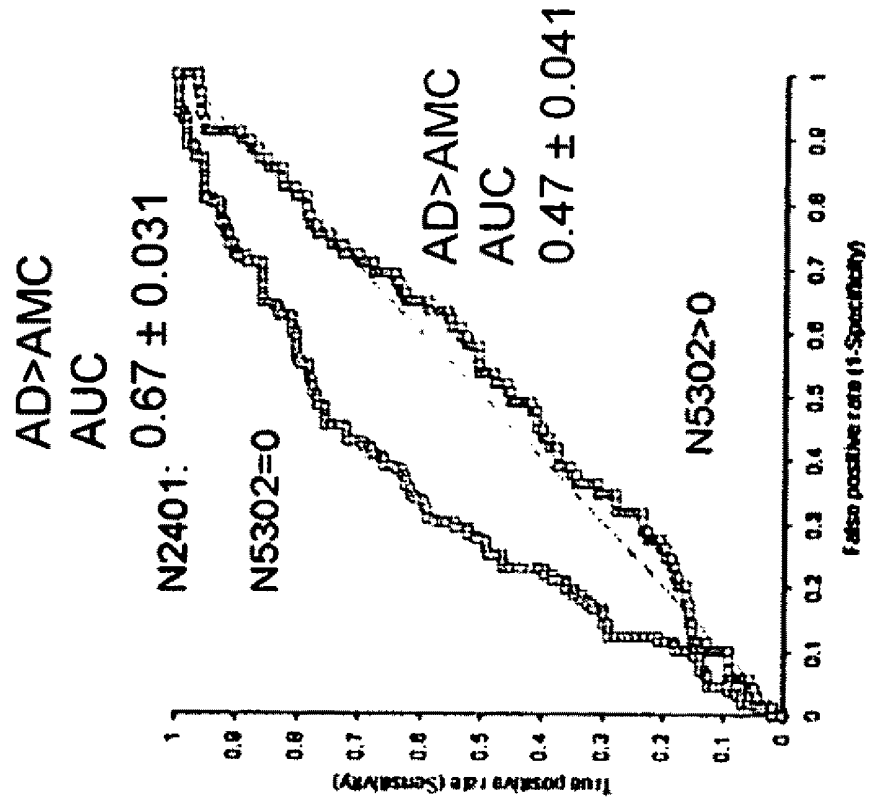


Fig. 24B

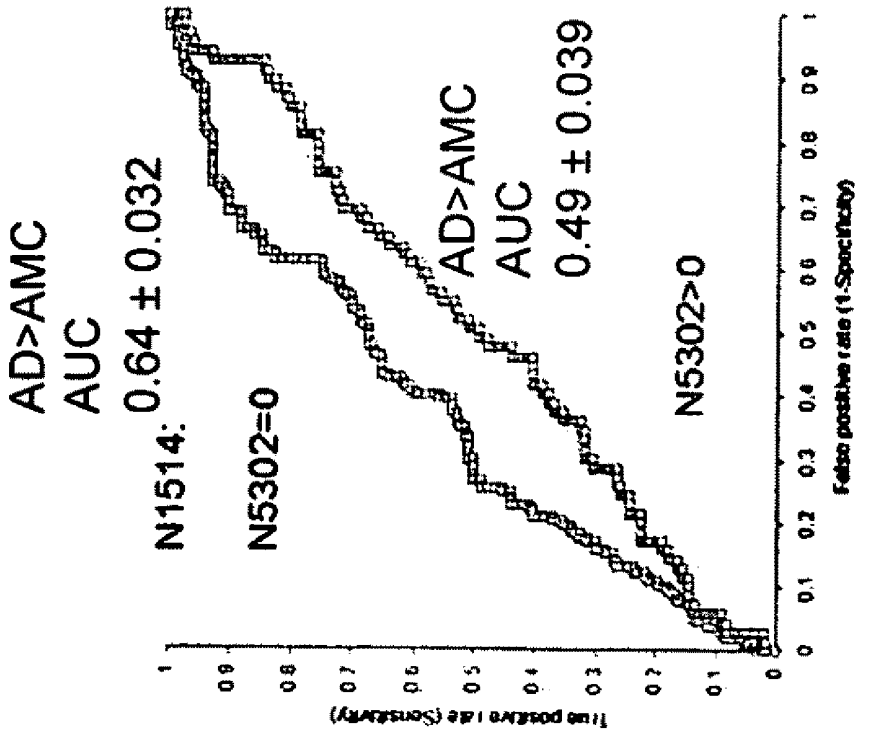


Fig. 24A

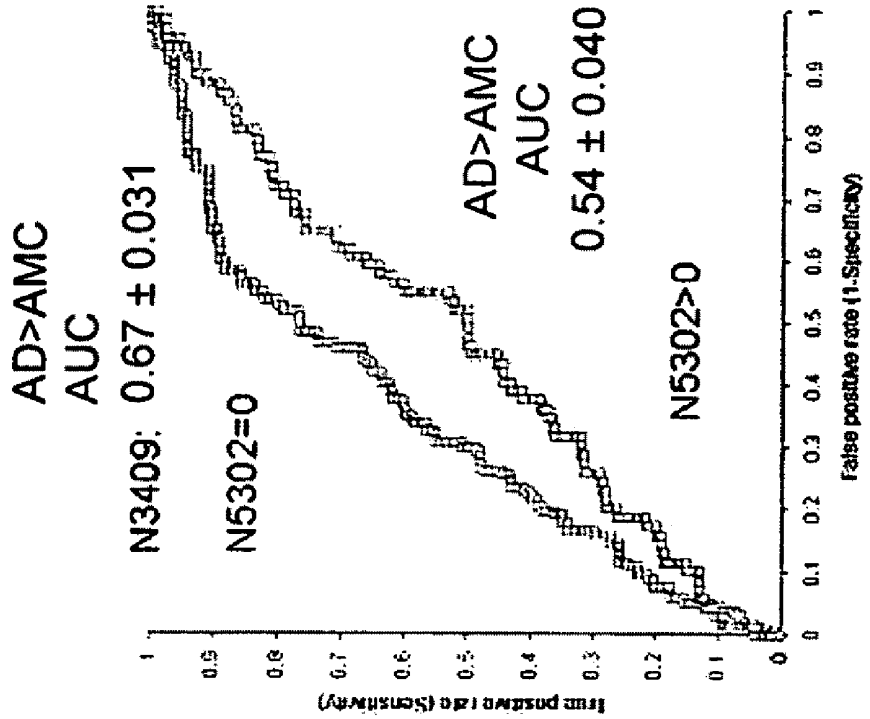


Fig. 24D

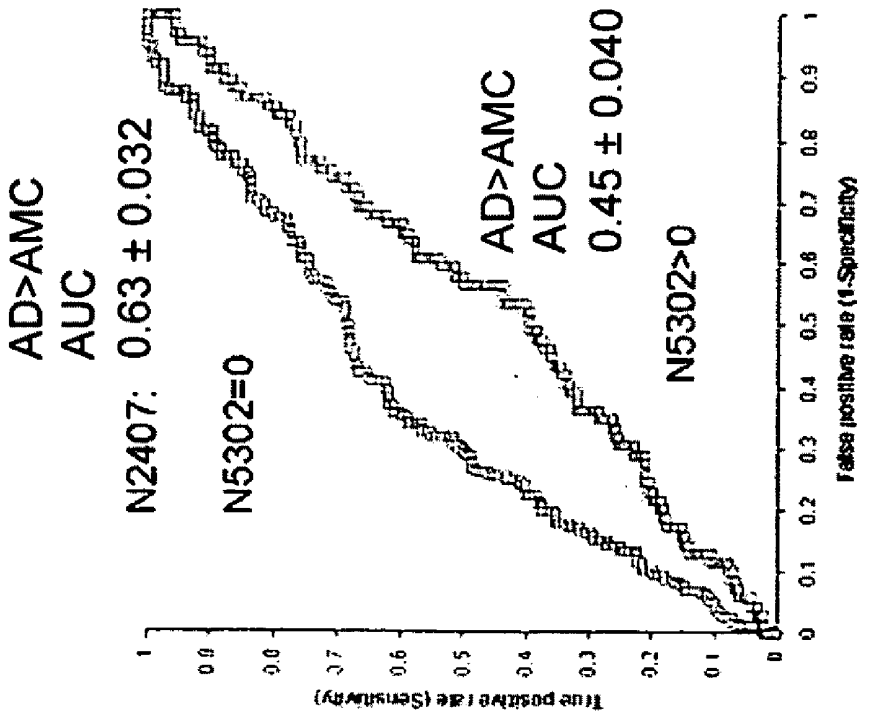


Fig. 24C

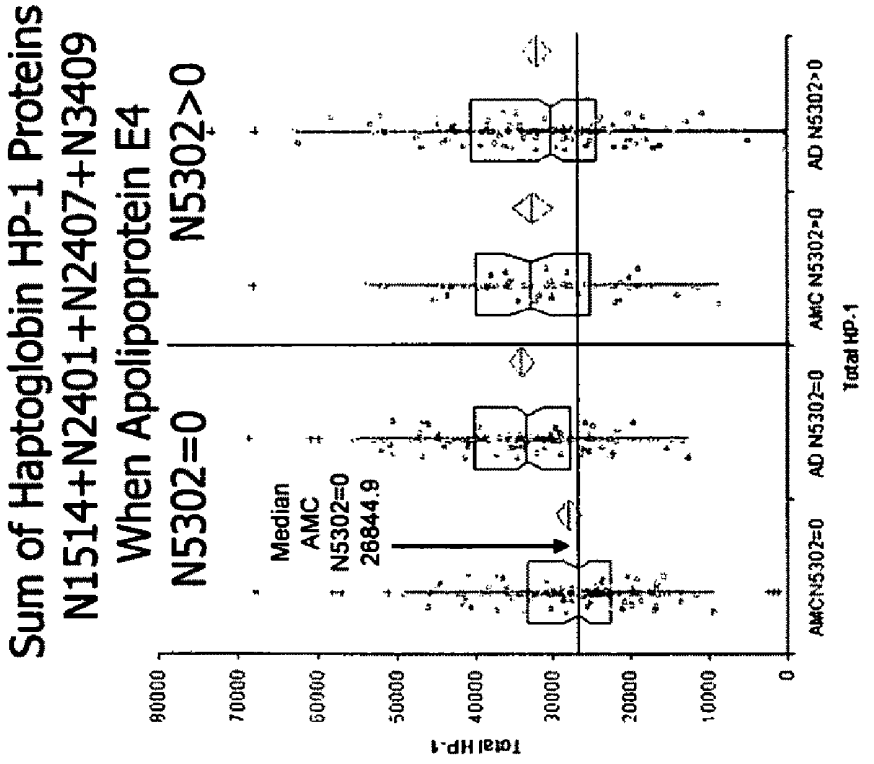


Fig. 25A

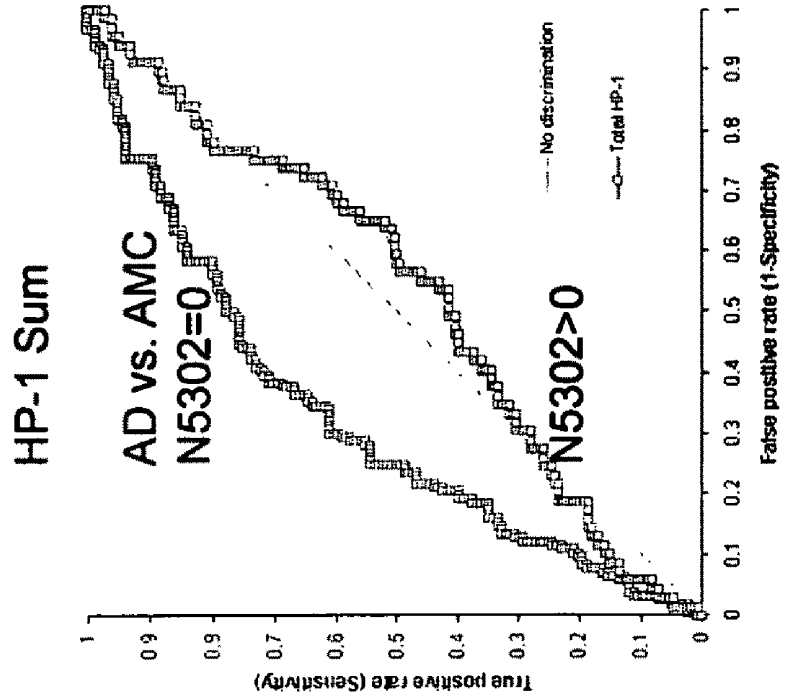


Fig. 25B

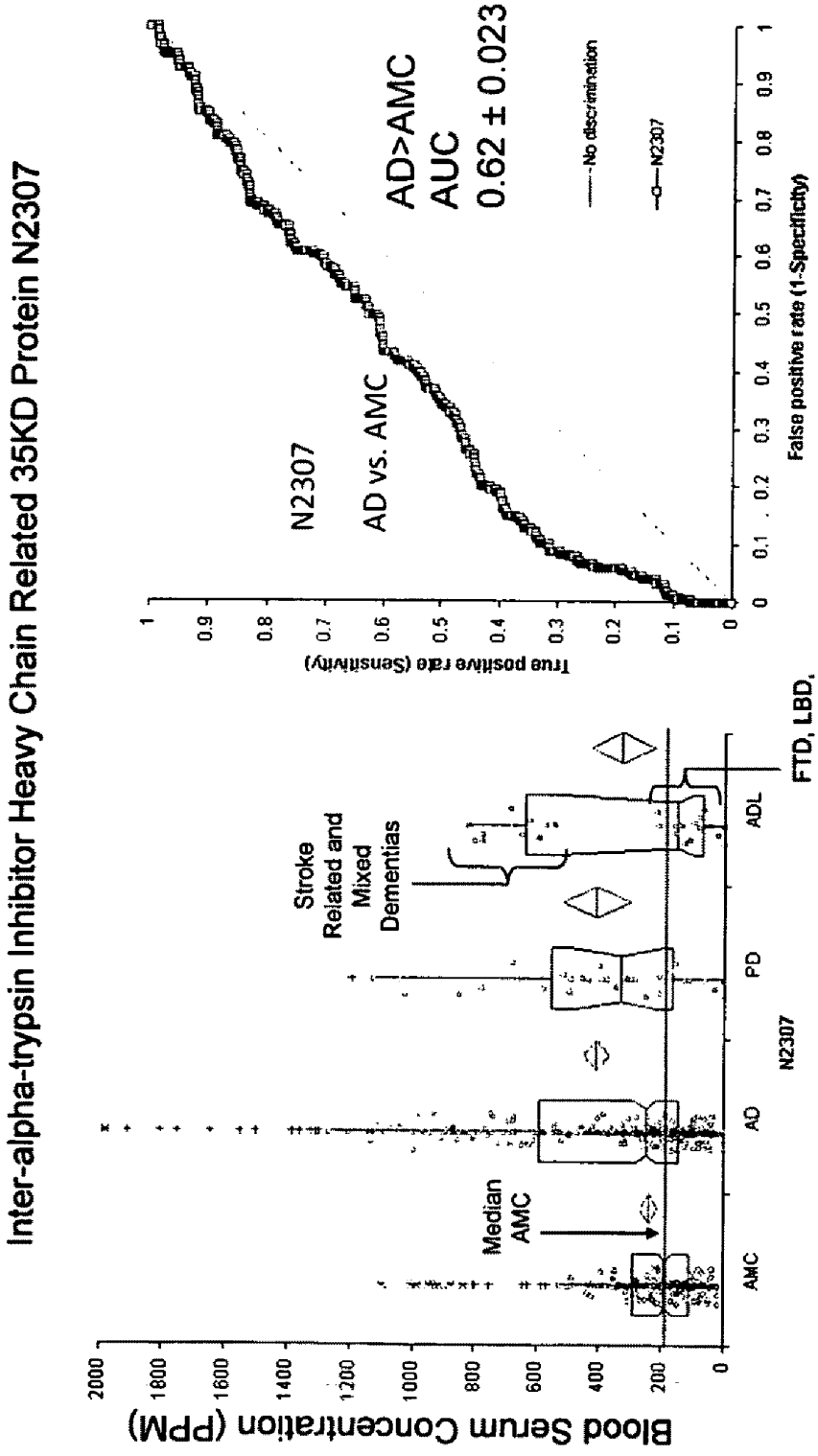


Fig. 26A

Fig. 26B

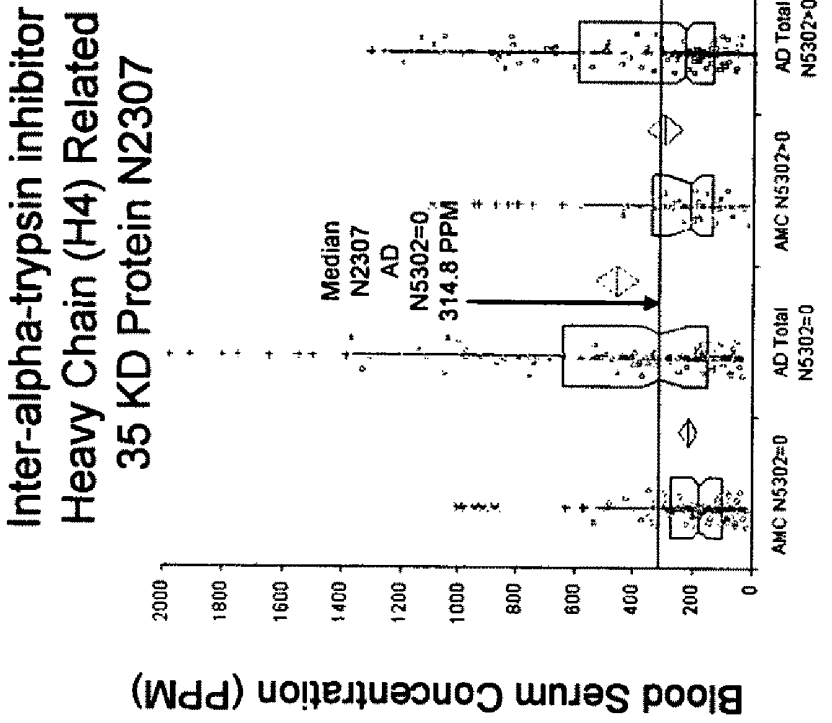


Fig. 27A

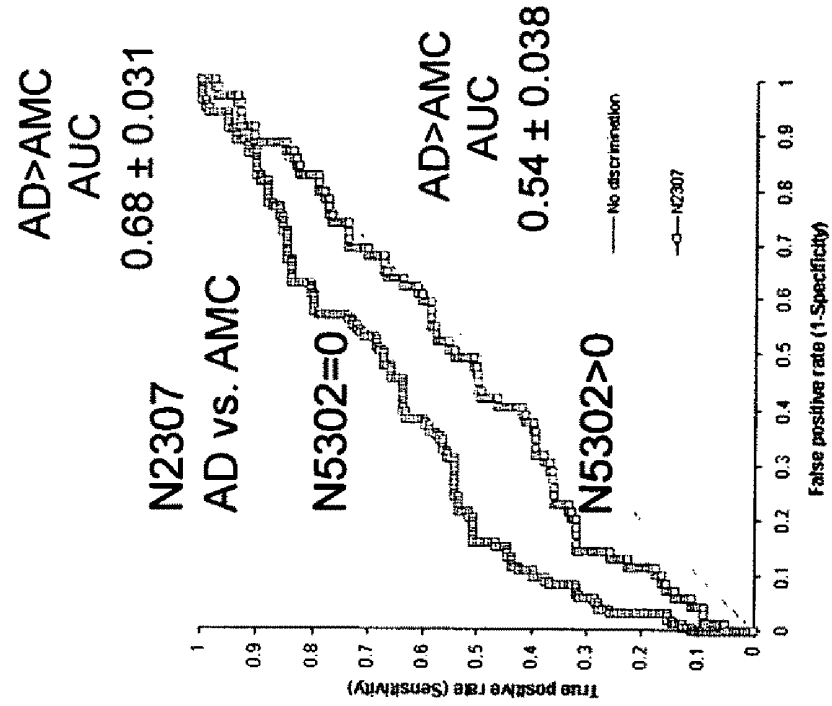


Fig. 27B

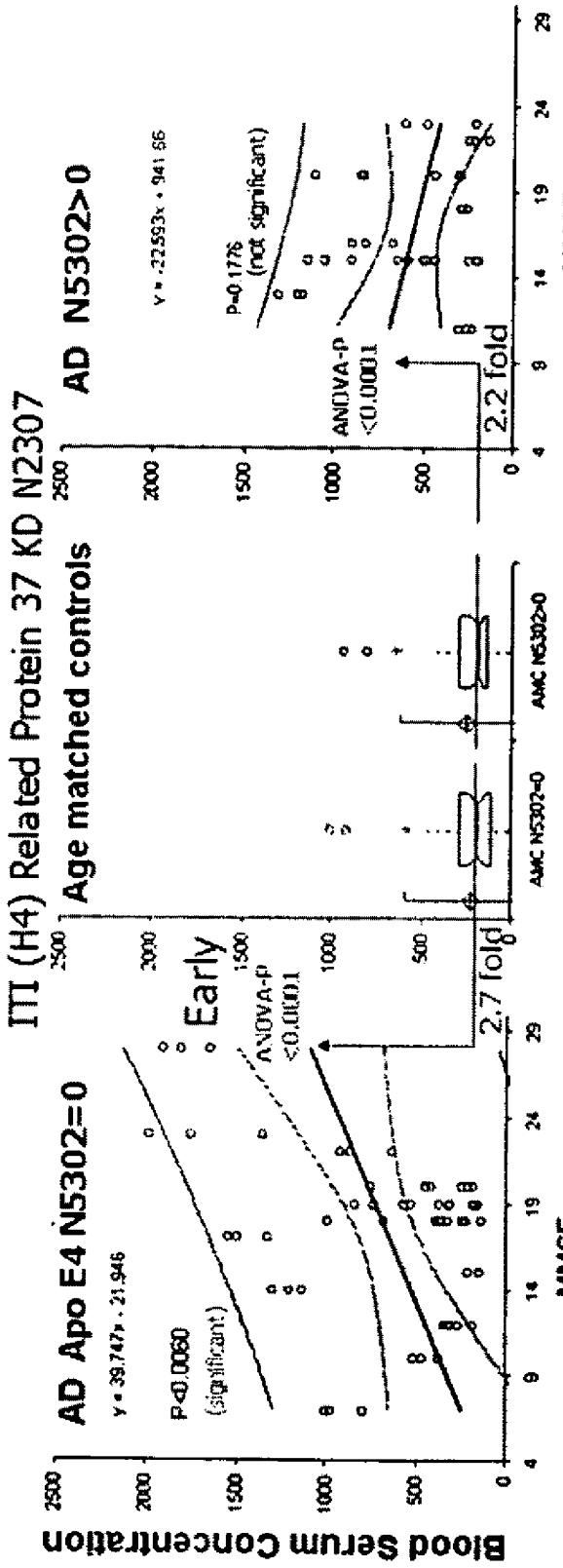


Fig. 28A

Fig. 28B

Fig. 28C

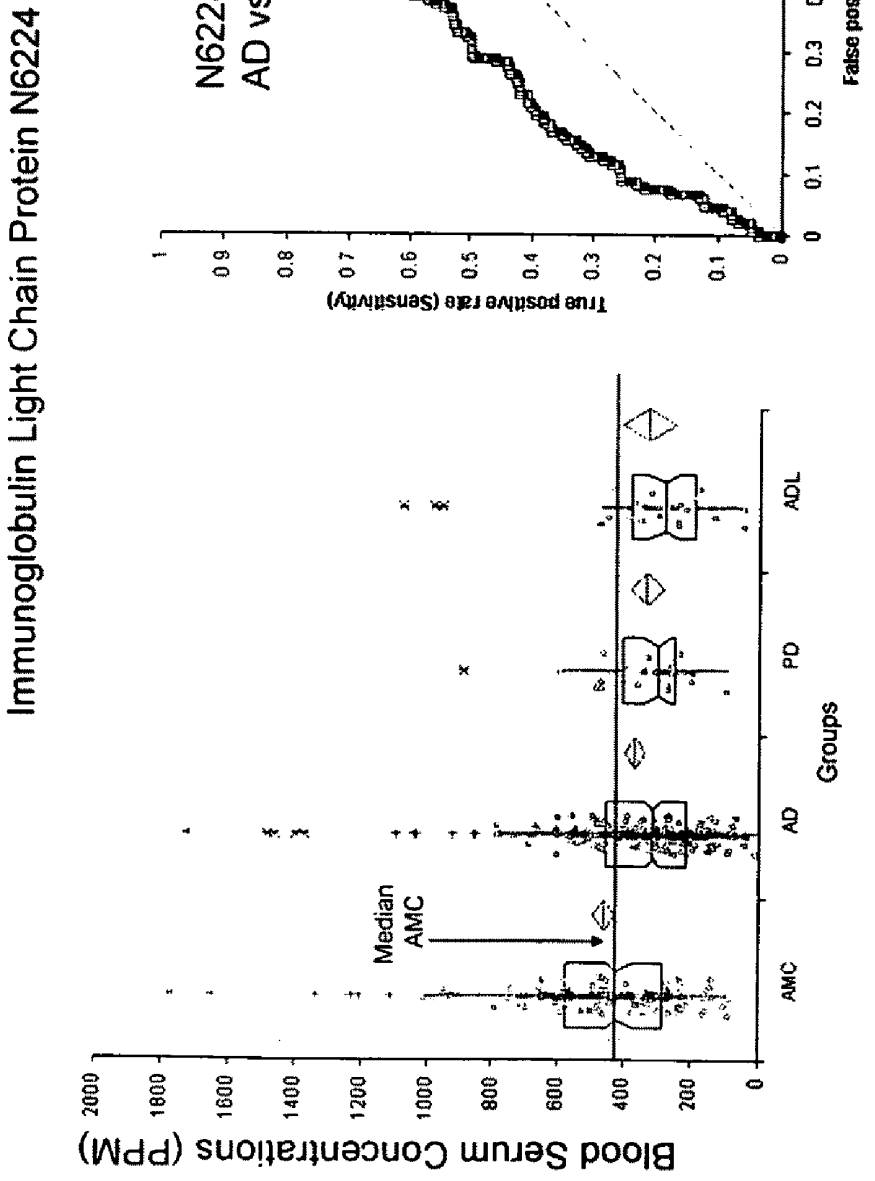


Fig. 29A

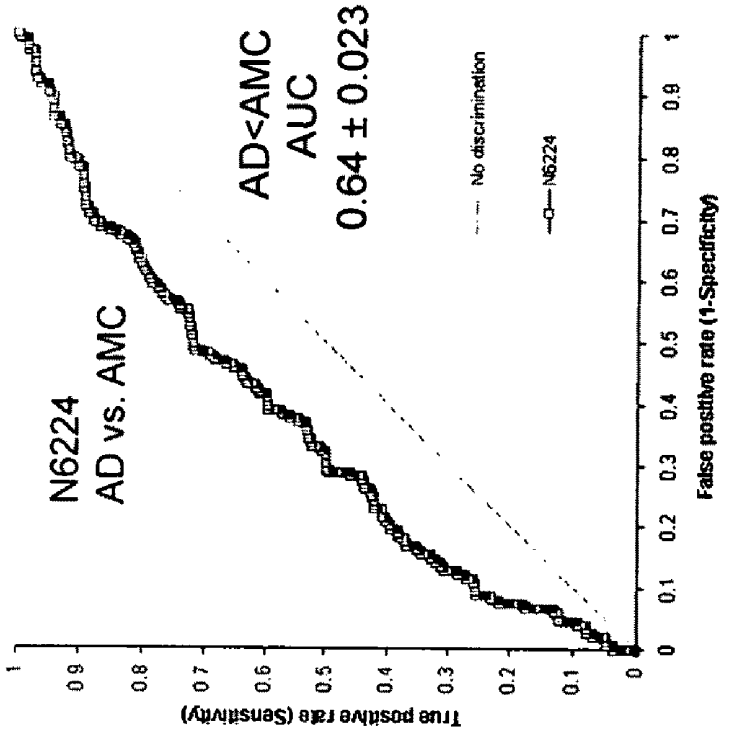


Fig. 29B

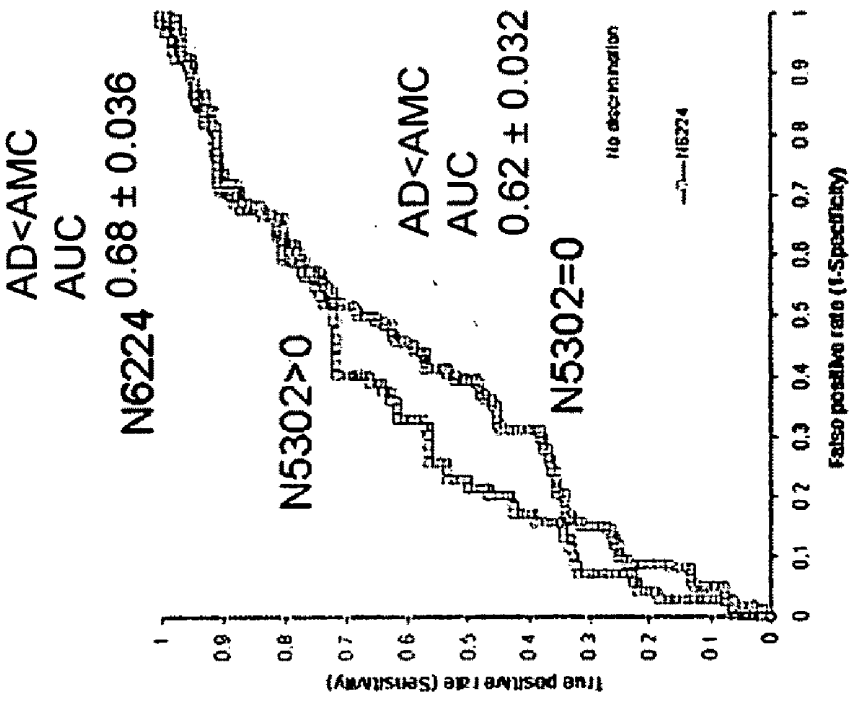


Fig. 30B

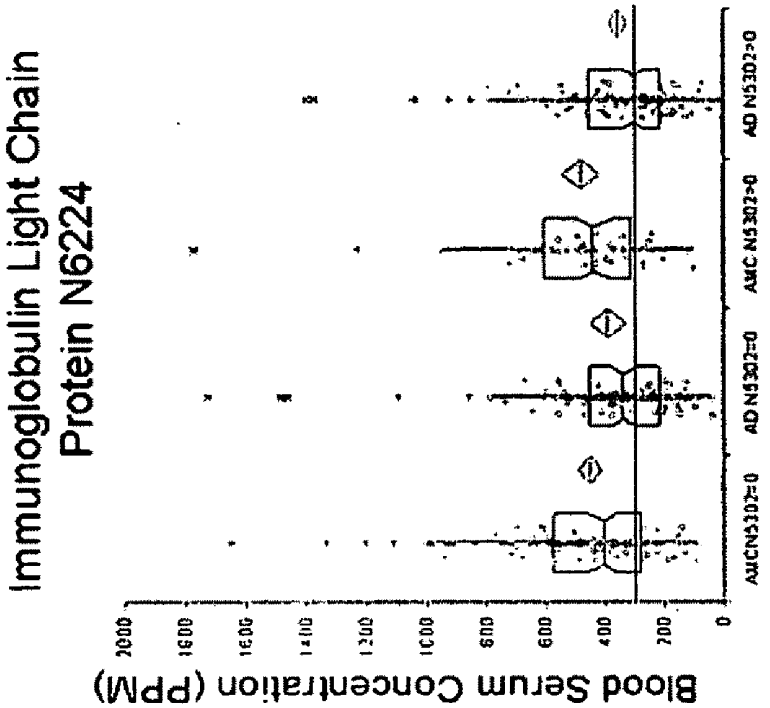


Fig. 30A

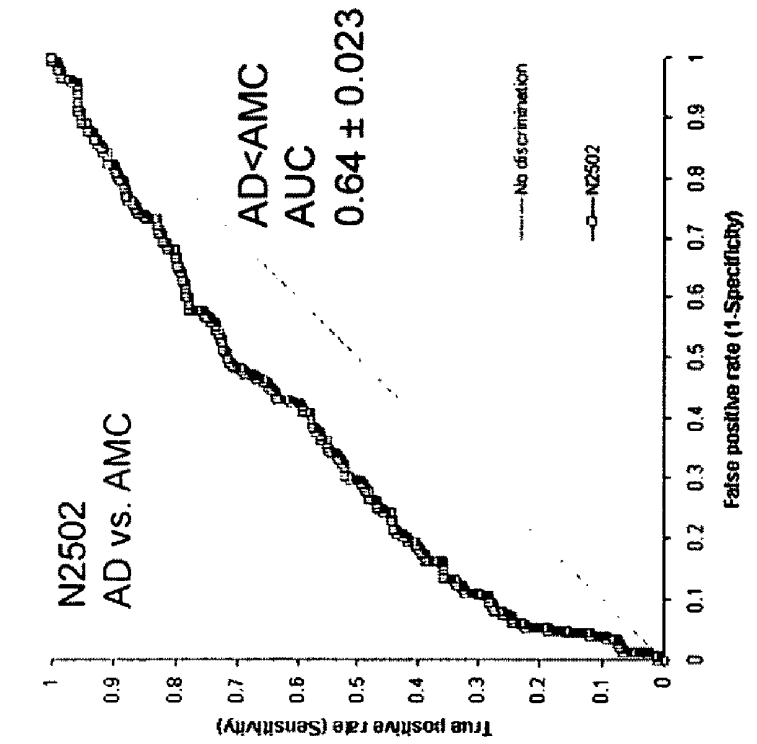


Fig. 31B

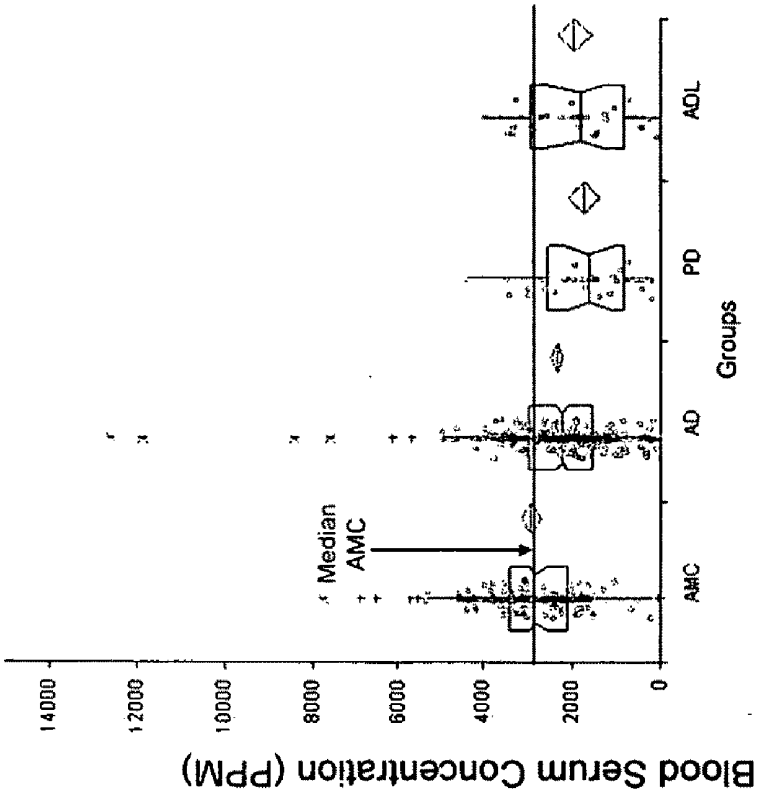


Fig. 31A

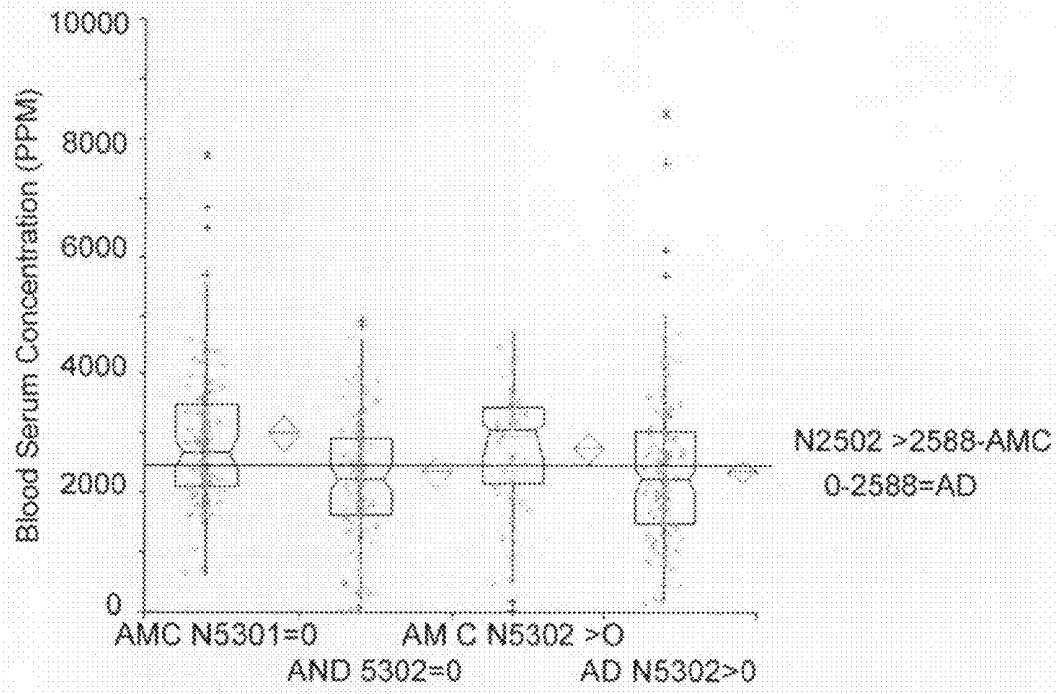


Fig. 32A

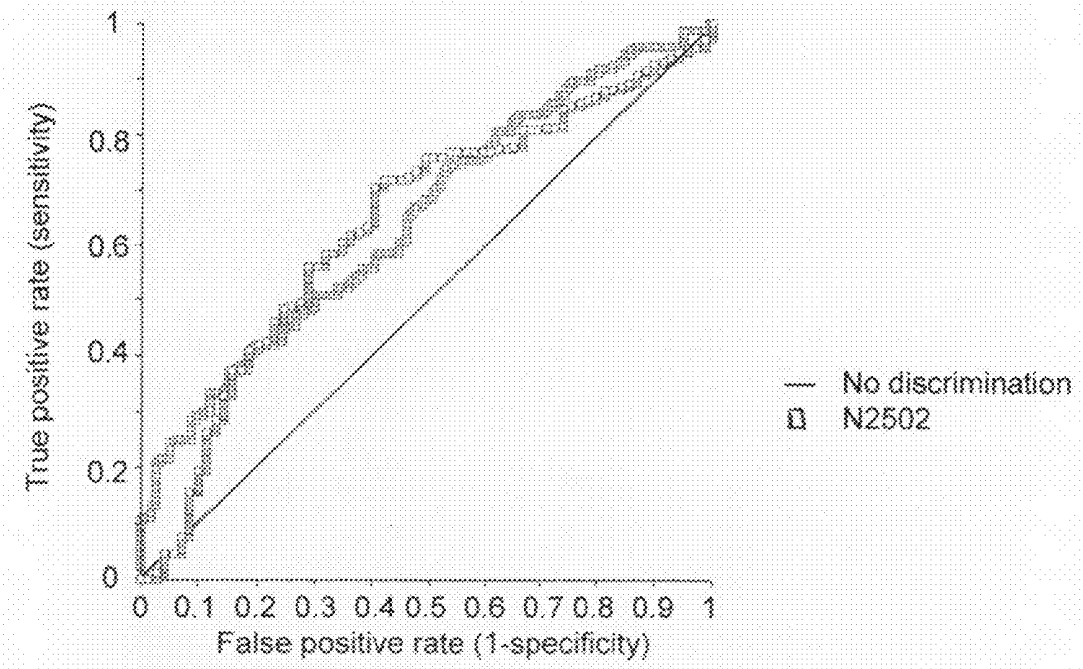


Fig. 32B

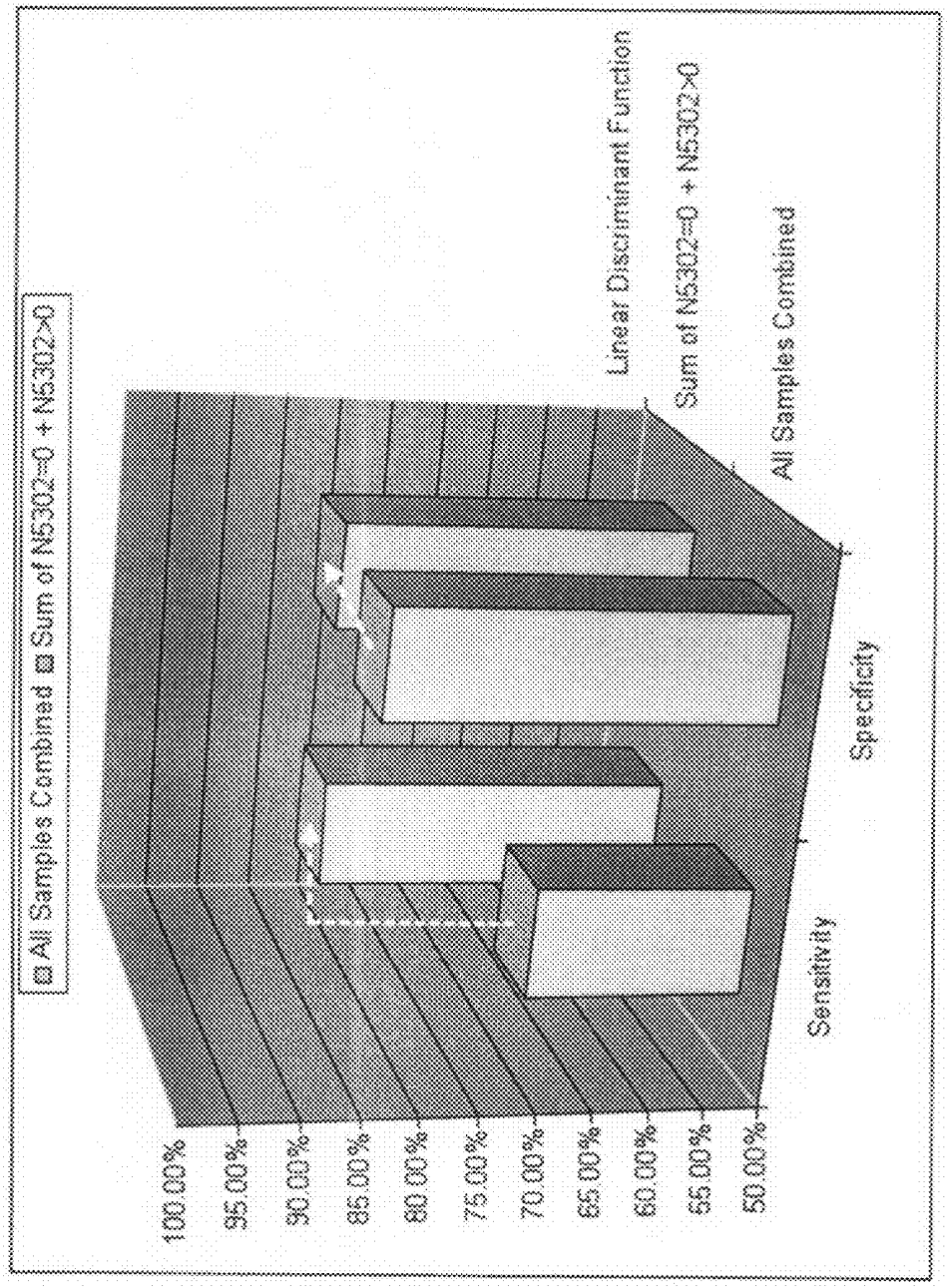

All Samples Combined
Sum of N5302=0 + N5302>0
100.00%
95.00%
90.00%
85.00%
80.00%
75.00%
70.00%
65.00%
60.00%
55.00%
50.00%
Sensitivity
Specificity
All Samples Combined
Sum of N5302=0 + N5302>0
Linear Discriminant Function

Fig. 33

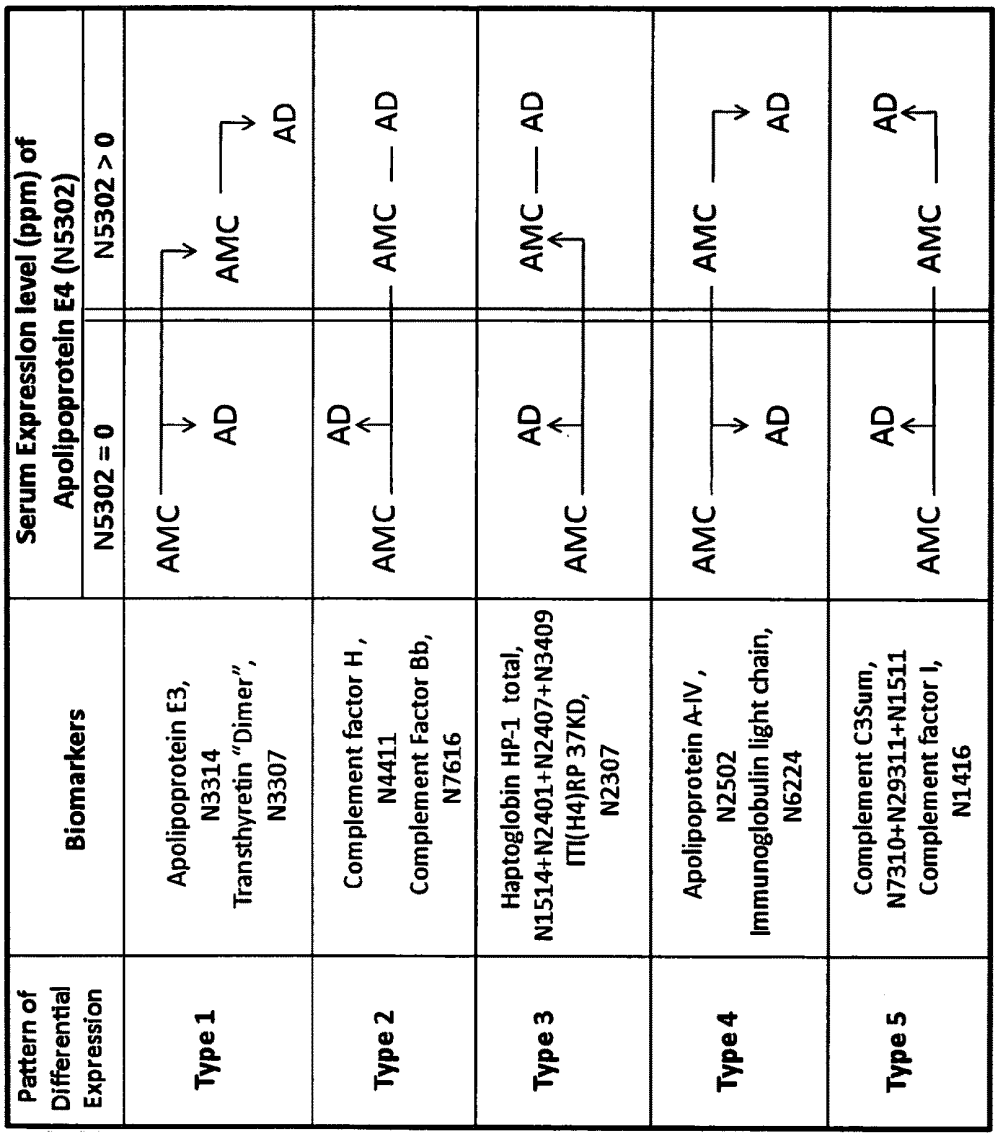

Pattern of Differential Expression
Biomarkers
Serum Expression level (ppm) of Apolipoprotein E4 (N5302)
N5302 = 0
N5302 > 0
Type 1
Apolipoprotein E3, N3314
Transthyretin "Dimer", N3307
AMC
AD
AMC
AD
Type 2
Complement factor H, N4411
Complement Factor Bb, N7616
AD
AMC
AMC — AD
Type 3
Haptoglobin HP-1 total, N1514+N2401+N2407+N3409
ΠΙ(H4)RP 37KD, N2307
AD
AMC
AMC — AD
Type 4
Apolipoprotein A-IV, N2502
Immunoglobulin light chain, N6224
AMC
AD
AMC
AD
Type 5
Complement C3Sum, N7310+N29311+N1511
Complement factor I, N1416
AD
AMC
AD
AMC

Fig. 34

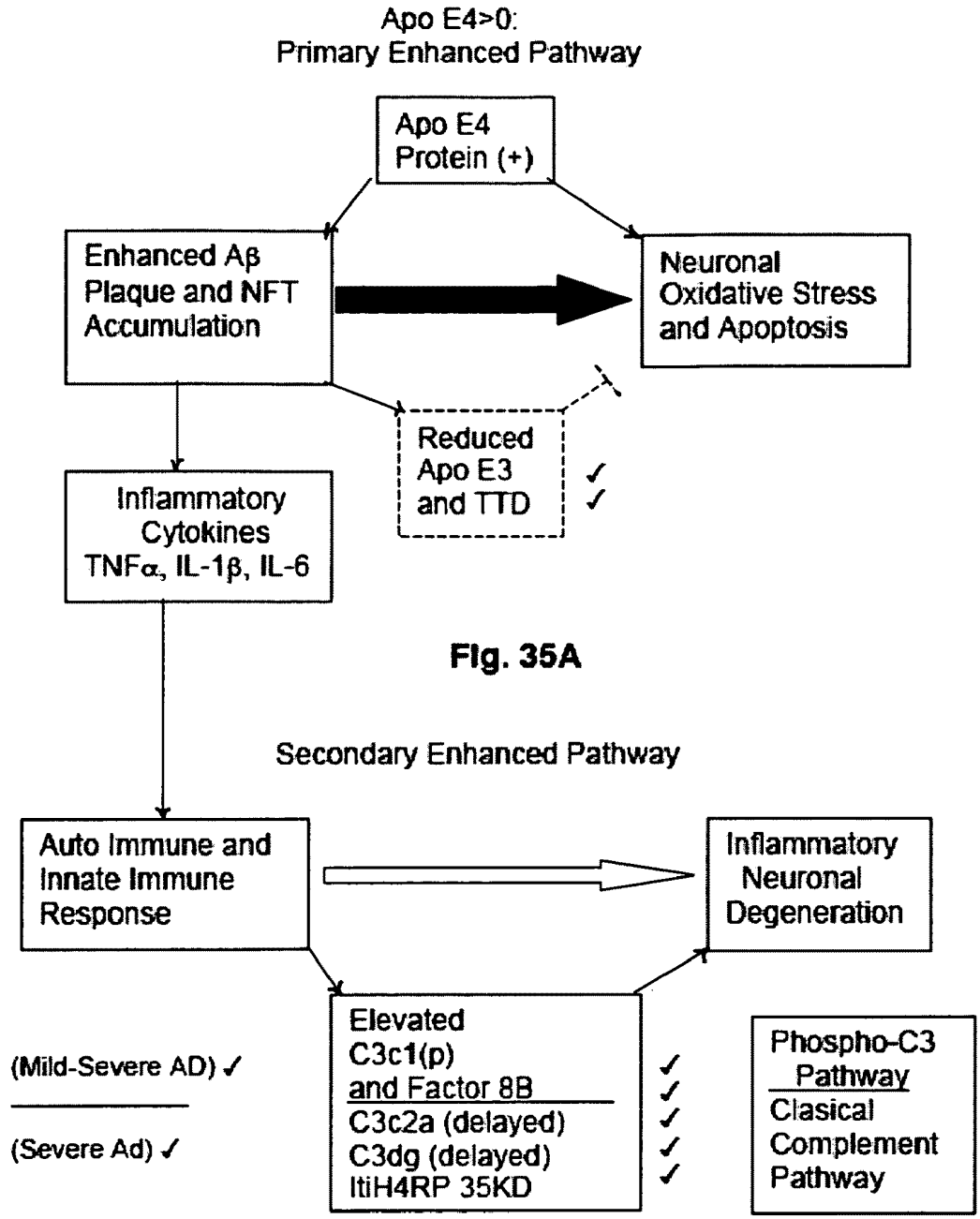


Fig. 35A

Fig. 35B

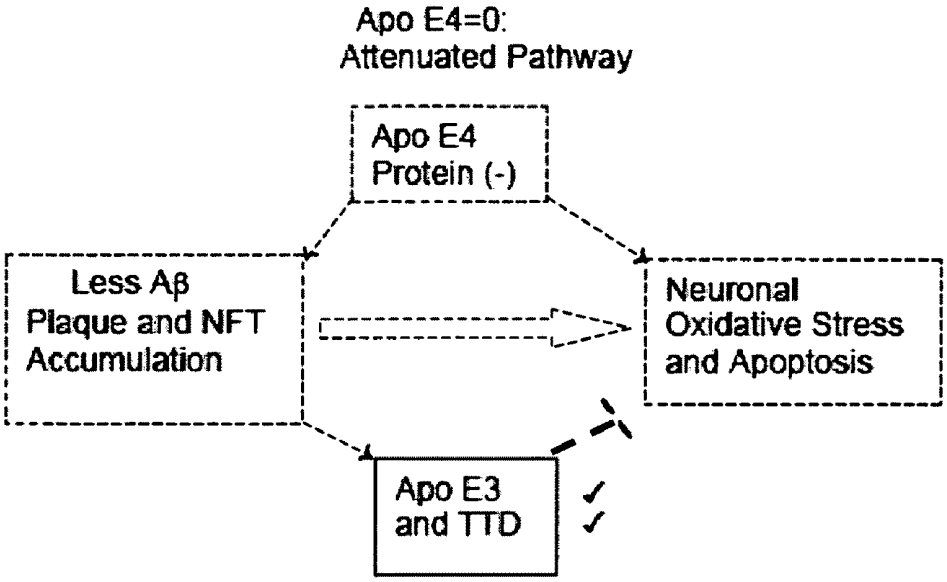
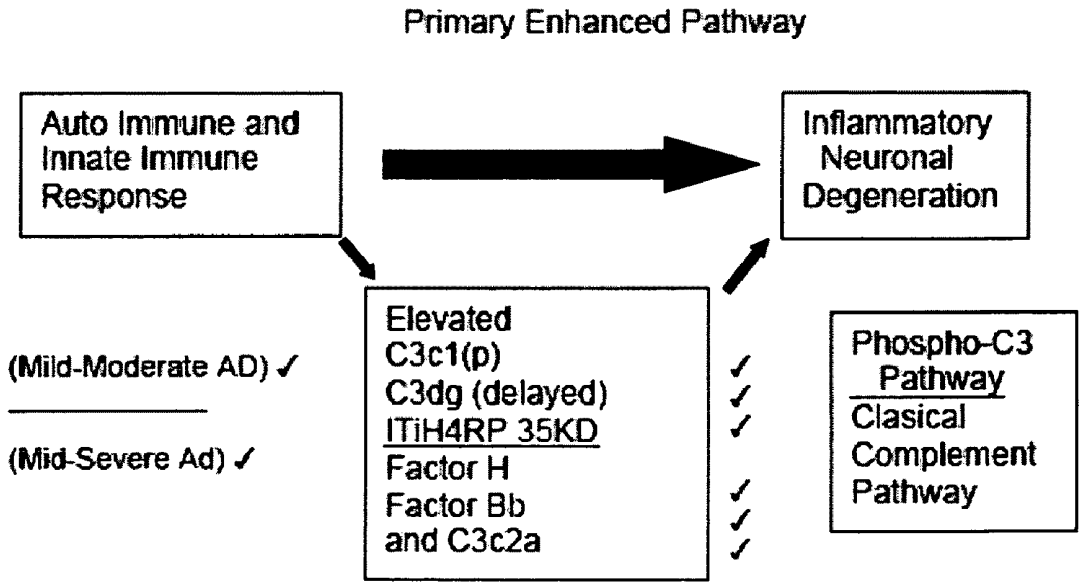


Fig. 35C



✓ From Blood Serum Biomarker Protein Concentration

Fig. 35D

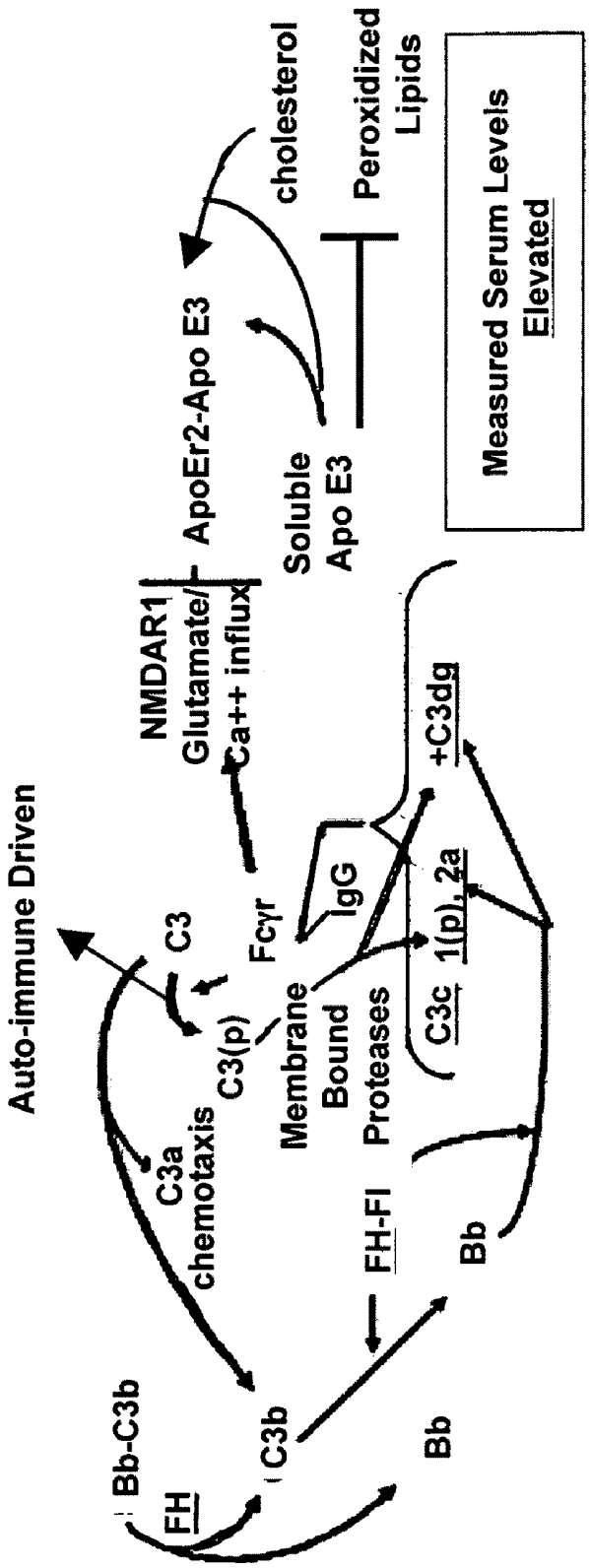

Auto-immune Driven
C3
C3(p)
C3a
chemotaxis
Bb-C3b
FH
C3b
Bb
FH-FI
Bb
Membrane
Bound
Proteases
C3c
1(p), 2a
Fcγr
IgG
+C3dg
NMDAR1
Glutamate/
Ca++ influx
ApoEr2-Apo E3
Soluble
Apo E3
cholesterol
Peroxidized
Lipids
Measured Serum Levels
Elevated

Fig. 36A

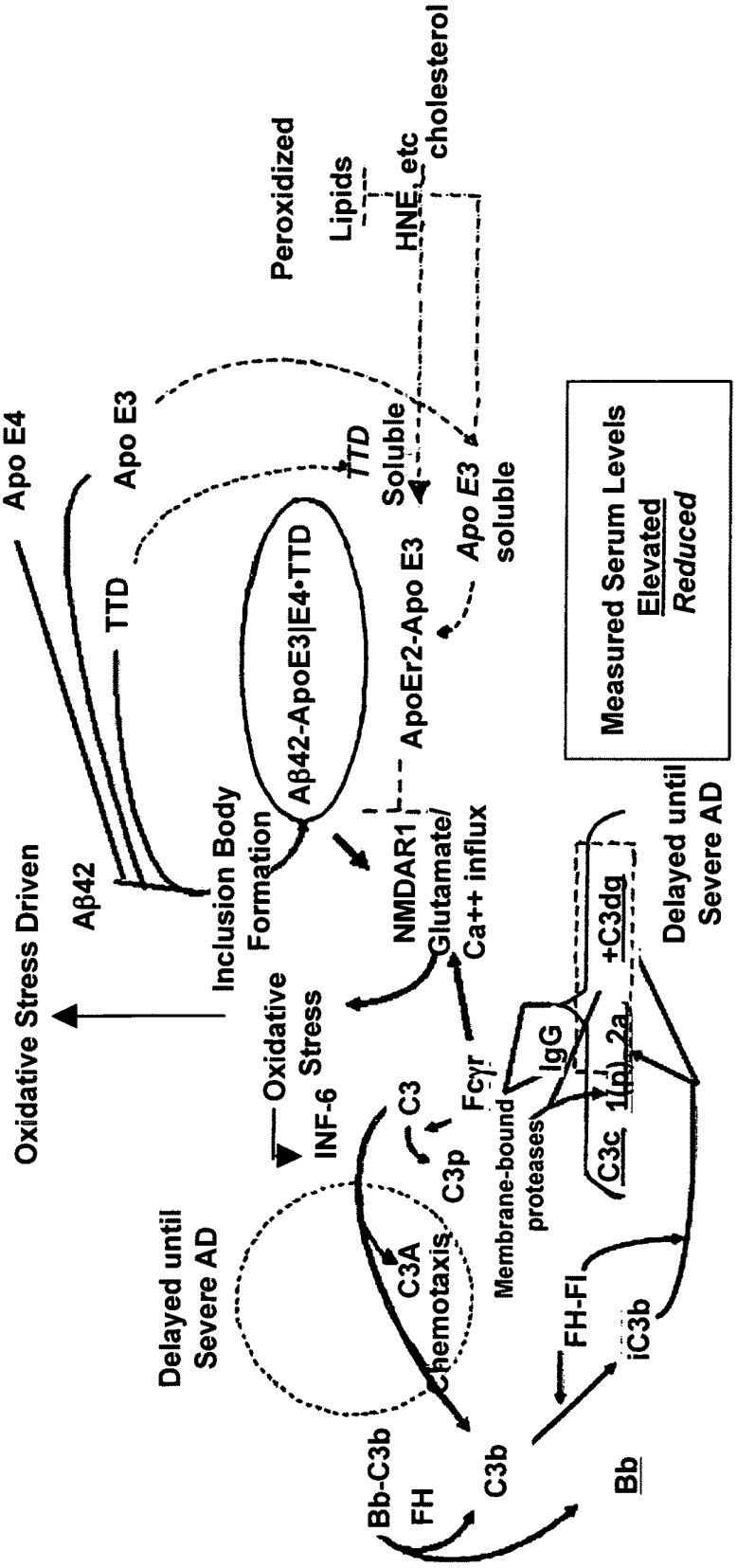

Oxidative Stress Driven
Aβ42
Inclusion Body Formation
Aβ42-ApoE3|E4•TTD
Apo E4
Apo E3
TTD
TTD
Soluble
Apo E3 soluble
ApoEr2-Apo E3
Peroxidized Lipids
HNE etc
cholesterol
NMDAR1
Glutamate/ Ca++ influx
Oxidative Stress
INF-6
C3
C3p
Fcγr
IgG
Membrane-bound proteases
C3c
1(α) 2a
+C3dg
Delayed until Severe AD
C3A Chemotaxis
FH-FI
iC3b
Bb-C3b
FH
C3b
Bb
Measured Serum Levels
Elevated
Reduced
Delayed until Severe AD

Fig. 36B

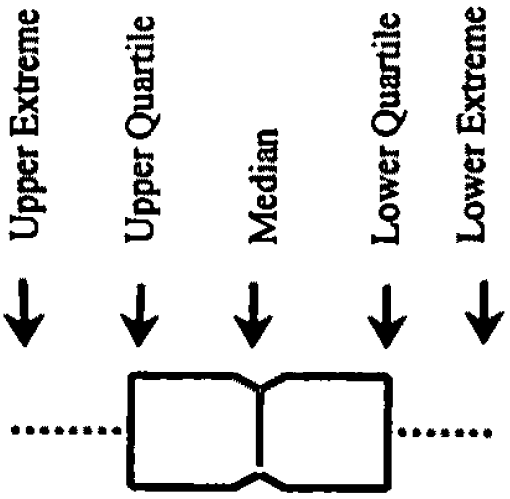

Upper Extreme
Upper Quartile
Median
Lower Quartile
Lower Extreme

Fig. 37

MULTIPLE FORMS OF ALZHEIMER'S DISEASE BASED ON DIFFERENCES IN CONCENTRATIONS OF PROTEIN BIOMARKERS IN BLOOD SERUM

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Utility Patent Application Ser. No. 11/503,881 filed Aug. 14, 2006 which claims priority to U.S. Provisional patent application Ser. No. 60/708,992 filed on Aug. 17, 2005, now abandoned.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] This invention relates to the identification of the relationships between two or more biomarkers for differential diagnosis of neurodegenerative disease. More specifically, the present invention relates to protein biomarkers for Alzheimer's disease, whereby lack of detection, and/or the quantity of a first protein biomarker in a biological sample from Alzheimer's disease patients correlates with significant differences in the quantities of other protein biomarkers of Alzheimer's disease. When Alzheimer's disease patients and age-matched normal control subjects are each placed into separate categories based on whether they do or do not have detectable quantities of the first protein biomarker, the protein identities of, and the differences in the quantities of the first protein biomarker and/or one or more other protein biomarkers in the biological sample provide opportunities: improve sensitivity and specificity of differential diagnosis; measure disease severity and monitor drug response; monitor drug clinical trial stratification of patients; indicate differences in neuronal degeneration mechanisms in the patients; measure the activity of these mechanisms of neuronal degeneration; determine which of these mechanisms of neuronal degeneration predominates; determine which biomarkers and disease mechanisms measure the severity of Alzheimer's disease in the patients; discover new targets for drug therapies; and develop companion diagnostics.

[0004] More particularly, the present invention relates to the identification of the relationships between two or more of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor heavy chain (H4) related protein, as biomarkers for distinguishing between different categories or types of Alzheimer's disease, and for early detection, screening, diagnosis, differential diagnosis, and monitoring of disease severity and disease mechanisms of patients with Alzheimer's disease (AD), Alzheimer's disease Like (AD-Like) dementias, Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease), and Parkinson's disease.

[0005] 2. Description of the Related Art

[0006] Proteomics is a new field of medical research wherein the proteins of an organism, including a human being are studied as a group, are identified, and linked to biological functions, including roles in a variety of disease states. With the completion of the mapping of the human genome, the identification of unique gene products, or proteins, has increased exponentially. In addition, molecular diagnostic testing for the presence of certain proteins already known to be involved in certain biological functions has progressed from research applications alone to use in disease screening and diagnosis for clinicians. However, proteomic testing for diagnostic purposes remains in its infancy. There is, however, a great deal of interest in using proteomics for the elucidation of potential disease biomarkers and their uses in diagnosis and treatment of diseases.

[0007] Detection of abnormalities in the genome, including genetic mutations and minor genetic variants, can reveal the risk or potential risk for individuals to develop a disease. The transition from such risk to the emergence of disease can be characterized as an expression of genomic abnormalities or other abnormalities, not of genetic origin, in the proteome, i.e. in proteins. Thus, the appearance of abnormalities in the proteome signals the beginning of the process of cascading effects that can result in the deterioration of the health of the patient. Therefore, detection of proteomic abnormalities at an early stage is desirable in order to allow for detection of disease either before it is established or in its earliest stages where treatment may be most effective.

[0008] Recent progress using a novel form of mass spectrometry called surface enhanced laser desorption and ionization time of flight (SELDI-TOF) for the testing of ovarian cancer has led to an increased interest in proteomics as a diagnostic tool (Petricoin E F, et al). Furthermore, proteomics has been applied to the study of breast cancer through use of 2D gel electrophoresis and image analysis to study the development and progression of breast carcinoma in patients (Kuerer, H M, et al.).

[0009] Detection of biomarker molecules is an active field of research. For example, U.S. Pat. No. 5,958,785 discloses a biomarker for detecting long-term or chronic alcohol consumption. The biomarker disclosed is a single biomarker and is identified as an alcohol-specific ethanol glycoconjugate. U.S. Pat. No. 6,124,108 discloses a biomarker for mustard chemical injury. The biomarker is a specific protein band detected through gel electrophoresis and the patent describes use of the biomarker to produce protective antibodies in a kit to identify the presence or absence of the biomarker in individuals who may have been exposed to mustard poisoning. U.S. Pat. No. 6,326,209 discloses measurement of total urinary 17 ketosteroid-sulfates as biomarkers of biological age. U.S. Pat. No. 6,693,177 discloses a process for preparation of a single biomarker specific for 0-2 acetylated sialic acid and useful for diagnosis and outcome monitoring in patients with lymphoblastic leukemia.

[0010] Neurodegenerative diseases such as Alzheimer's disease (AD) are difficult to diagnose, particularly in their earlier stages. Currently there are no biomarkers in blood available for early diagnosis, differential diagnosis, determination and monitoring of disease severity and mechanisms, or for use as drug targets for treatment of neurodegenerative diseases such as Alzheimer's disease.

[0011] Therefore, there remains a need for better ways to objectively and accurately detect, diagnose, and distinguish AD from other neurodegenerative diseases, to accurately and specifically diagnose patients, to predict therapeutic response, to stratify patients for clinical trials, to measure disease severity, to monitor patient's response to treatment, and to find new drug targets to design new drugs.

[0012] In Alzheimer's disease, one genetic abnormality, the dementia risk Apo E ϵ4 gene allele, is inherited as one of three Apo E alleles, termed ϵ2, ϵ3, and ϵ4, with mean frequencies in

the general population of about 8%, 78%, and 14%, respectively (Utermann G, et al.). The degree of risk of dementia conferred by the Apo E ϵ4 allele rises in a "gene dose" dependent manner (Corder, E. H. et al.), increasing with the number of Apo E ϵ4 alleles inherited, from: zero, i.e. Apo E ϵ4 non-carriers; to carriers of one Apo E ϵ4 allele, i.e. ϵ4/ϵ3; ϵ4/ϵ2 hetero-zygotes; to two Apo E ϵ4 alleles, i.e. zygotes (Greenwood P M, et al.), all of whom are capable of developing Alzheimer's disease, although those lacking the Apo E ϵ4 allele may tend to get the disease at a later age of onset (Poirier J, J.).

SUMMARY OF THE INVENTION

[0013] The present invention relates to blood serum protein biomarkers for Alzheimer's disease, whereby the detection and/or concentration, or the lack of detection of one or more proteins correlates with significant increases or decreases in one or more other proteins in a disease specific manner. More specifically, the present invention relates to blood serum protein biomarkers for Alzheimer's disease, whereby the detection, and/or concentration, or the lack of detection, of a first biomarker such as an Apolipoprotein E4 protein in the blood serum of Alzheimer's disease patients correlates with significant differences in the blood serum concentrations of additional protein biomarkers of Alzheimer's disease, such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein. Also in the present invention, Alzheimer's disease patients, and age-matched normal control subjects, are each placed into separate categories based on whether they do or do not have detectable blood serum levels of a first biomarker such as an Apolipoprotein E4 protein, and the differences in these and other Alzheimer's disease blood serum biomarker protein profiles indicate differences in Alzheimer's disease mechanisms, providing opportunities for improvements in differential diagnosis, disease severity and drug response monitoring, drug clinical trial stratification of patients, and for discovery of new targeted therapies.

[0014] One aspect of the present invention is the use of blood serum protein biomarkers for screening, diagnosis, differential diagnosis, and determining and monitoring of disease severity and mechanisms of Alzheimer's disease, comprising obtaining a blood serum sample from a test subject; determining whether a quantity of an Apolipoprotein E4 protein can be detected in the blood serum sample, wherein detection of a quantity of a first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of one form of Alzheimer's disease or a normal condition with a potential to develop that form of Alzheimer's disease, and the lack of detection of a quantity of a first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of another form of Alzheimer's disease or a normal condition with a potential to develop the other form of Alzheimer's disease.

[0015] Yet another aspect of the present invention is the use of the blood serum protein biomarkers for screening, diagnosis, or differential diagnosis of Alzheimer's disease comprising obtaining a blood serum sample from a test subject; determining whether or not a quantity of a first protein biomarker such as an Apolipoprotein E4 protein can be detected in the blood serum sample; and determining the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample, and determining whether the first protein biomarker such as an Apolipoprotein E4 protein can be detected and determining the quantities of a first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in blood serum samples from normal control individuals, from patients with Alzheimer's disease, with Parkinson's disease, and with AD-Like and Mixed dementias, wherein the detection of the first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of one form of Alzheimer's disease or a normal condition with a potential to develop that form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject within the ranges of that form of Alzheimer's disease values is indicative of the presence of that form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject outside the range of that form of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an AD-Like or Mixed dementia, such as: Frontotemporal dementia (FTD); Lewy body dementia (LBD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's dis-

ease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA, and wherein the lack of detection of a quantity of the first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of another form of Alzheimer's disease or a normal condition with a potential to develop that other form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject within the ranges of that other form of Alzheimer's disease values is indicative of the presence of that other form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject outside the range of that other form of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an AD-Like or Mixed dementia, such as: Frontotemporal dementia (FTD); Lewy body dementia (LBD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA.

[0016] Yet another aspect of the present invention is the use of the blood serum protein biomarkers for screening, diagnosis, or differential diagnosis of Alzheimer's disease comprising obtaining a blood serum sample from a test subject; determining whether or not an Apolipoprotein E4 protein can be detected in the blood serum sample; and determining the quantity of a first protein biomarker such as Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample, by quantitative two-dimensional gel electrophoresis; and determining whether a quantity of the first protein biomarker such as an Apolipoprotein E4 protein can be detected, and quantitating the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Albumin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the protein expression patterns of the 2D gels of the serum samples; wherein the detection of the first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of one form of Alzheimer's disease or a normal condition with a potential to develop that form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject within the ranges of that form of Alzheimer's disease values is indicative of the presence of that form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject outside the range of that form of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an AD-Like or Mixed dementia, and wherein the lack of detection of a quantity of the first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of another form of Alzheimer's disease or a normal condition with a potential to develop that other form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject within the ranges of that other form of Alzheimer's disease values is indicative of the presence of that other form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a

Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject outside the range of that other form of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an AD-Like or Mixed dementia, such as: Frontotemporal dementia (FTD); Lewy body dementia (LBD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA.

[0017] Yet another aspect of the present invention is the use of the blood serum protein biomarkers for screening, diagnosis, or differential diagnosis of Alzheimer's disease comprising obtaining a blood serum sample from a test subject; determining whether or not a first protein biomarker such as an Apolipoprotein E4 protein can be detected in the blood serum sample; and determining the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of one or more additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample, by an immunoassay using an antibody that recognizes the first protein biomarker such as an Apolipoprotein E4 protein and one or more other antibodies that recognize one or more additional protein biomarkers such as an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein, in the blood serum sample of the test subject, wherein the detection of the first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of one form of Alzheimer's disease or a normal condition with a potential to develop that form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a

[0018] Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject within the ranges of that form of Alzheimer's disease values is indicative of the presence of that form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject outside the range of that form of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an AD-Like or Mixed dementia, such as: Frontotemporal dementia (FTD); Lewy body dementia (LBD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA, and wherein the lack of detection of a quantity of the first protein biomarker such as an Apolipoprotein E4 protein in the test subject blood serum sample is indicative of another form of Alzheimer's disease or a normal condition with a potential to develop that other form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject within the ranges of that other form of Alzheimer's disease values is indicative of the presence of that other form of Alzheimer's disease, and the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample of the test subject outside the range of that other form of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an AD-Like or Mixed dementia, such as: Frontotemporal dementia (FTD); Lewy body dementia (LBD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined

with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA.

[0019] Yet another aspect of the present invention is the use of blood serum protein biomarkers, for early detection and for monitoring the disease severity and response to therapy of patients with Alzheimer's disease, comprising obtaining a blood serum sample from a test subject; determining whether a first protein biomarker such as an Apolipoprotein E4 protein can be detected and determining the quantity of the first protein biomarker such as an Apolipoprotein E4 protein, and of additional protein biomarkers such as an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample from the test subject, and in blood serum samples from normal control individuals, and from patients with mild (MMSE score=25-20), moderate (MMSE score=19-11) and severe (MMSE≦10) Alzheimer's disease, wherein, whether an Apolipoprotein E4 protein can be detected and the quantity of an Apolipoprotein E4 protein, and of additional protein biomarkers such as an the first protein biomarker such as Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a complement C3dg protein, a Complement Factor Bb protein, a Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein in the blood serum sample from the test subject, indicates the degree of severity of Alzheimer's disease in the test subject.

[0020] The foregoing has outlined rather broadly several aspects of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and the specific embodiment disclosed might be readily utilized as a basis for modifying or redesigning the structures for carrying out the same purposes as the invention. It should be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] For a more complete understanding of the present invention, and the advantages thereof, reference is now made to the following descriptions taken in conjunction with the accompanying drawings, in which:

[0022] FIGS. **1**A-**1**B illustrate the dynamic range of the assay and the reproducibility within triplicate assays of quantitative 2D gel electrophoresis of human blood serum. Shown in FIG. **1**A are several protein spots (circles) within the 2D gel pattern of human blood serum, with spot concentrations ranging from 55 ppm to 15,789 ppm (white arrows). FIG. **1**B shows triplicate analysis (details of three 2D gels run with the same blood serum sample) with a coefficient of variation=of 13.8% for the triplicate analysis of the individual spot concentrations. See also Table 2 for reproducibility over the dynamic range.

[0023] FIG. **2** illustrates the location (circles and numbers) of biomarker protein spots within a 2D Gel electrophoresis protein expression profile of human blood serum, namely Apolipoprotein E4 protein spot N5302; Apolipoprotein E3 protein spot N3314; Transthyretin "Dimer" protein spot N3307; Complement C3c1 protein spot N7310; Complement C3c2a protein spot N9311; Complement C3dg protein spot N1511; Complement Factor Bb protein spot N7616; Complement Factor H/Hs protein spot N4411; Inter alpha Trypsin Inhibitor Heavy Chain H4 related 35 KD protein spot N2307; Immunoglobulin Light Chain protein spot N6224; Apolipoprotein A-IV protein spot N2502; Complement Factor I protein spot N1416: and Haptoglobin protein spots N1514, N2401, N2407, and N3409. These spots are among the differentially expressed proteins detected in 2D gels of blood serum collected from normal subjects, patients with neurodegenerative diseases and patients with like-disease disorders, where the indicated protein spots were identified by LC-MS/MS analysis of in-gel trypsin digests of the spots.

[0024] FIG. **3**A is a comparative statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel, illustrating the differential expression level (PPM) of an Apolipoprotein E4 protein spot N5302 in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, Neuro Exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P). FIG. **3**B shows receiver Operating Characteristic (ROC) curve of Apolipoprotein E4 spot N5302 when used as a single biomarker to differentiate between Alzheimer's disease patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.66±0.02, sensitivity, specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.

[0025] FIG. **4**A shows statistical Dot, Box and Whiskers graph constructed using Analyze-it software for Microsoft Excel, illustrating the differential expression level (PPM) of a Apolipoprotein E4 protein spot N5302 in blood serum, based on the quantitative 2D gel triplicate analysis data (dots) obtained with blood serum samples from age matched normal controls, and patients with Alzheimer's disease, (Total N5302 and N5302>0) constructed using Analyze-it software for Microsoft Excel. Blood serum samples were from: 75 Age matched normal control individuals (Controls); of which 23 Age matched normal control individuals (31%) had detectable quantities of Apolipoprotein E4 protein spot N5302 (N5302>0) in their blood serum; and 115 Alzheimer's disease patients (AD); of which 67 Alzheimer's disease patients (58%), had detectable quantities of Apolipoprotein E4 pro-

tein spot N5302 (N5302>0) in their blood serum. FIG. **4**B is Receiver Operating Characteristic (ROC) curve of Apolipoprotein E4 spot N5302 from populations where the biomarker level (ppm) was greater than zero (N5302>0) was used as a single biomarker to differentiate between 67 Alzheimer's disease (AD) patients and 23 age-matched control (AMC) subjects with an area under the curve (AUC) of 0.61±0.04.

[0026] FIG. **5**A is a comparative statistical Box and Whiskers graph constructed using Analyze-it software for Microsoft Excel, illustrating the differential expression level (PPM) of an Apolipoprotein E3 protein spot N3314 in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P). FIG. **5**B is a Receiver Operating Characteristic (ROC) curve of Apolipoprotein E3 spot N3314 when used as a single biomarker to differentiate between Alzheimer's disease (AD) patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.71±0.022, sensitivity, specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.

[0027] FIG. **6** shows a comparative statistical Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of an Apolipoprotein E3 protein spot N3314 in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from patients with Alzheimer's disease (AD) and age matched normal control (AMC) subjects, when Apolipoprotein E4 (spot N5302) was not detected (N5302=0, left panel) and when it was detected (N5302>0, right panel) in the 2D gels of their blood serum.

[0028] FIG. **7**A shows a plot of the Receiver Operator Characteristics (ROC) curve (calculated by using Analyse-it software for Microsoft Excel) of blood serum concentrations of Apolipoprotein E3 protein spot N3314 when used to distinguish between patients with Alzheimer's disease (AD) and age matched normal controls (AMC) as a function of whether Apolipoprotein E4 protein (spot N5302) is detected (N5302>0) or not detected (N5302=0) in blood serum. FIG. **7**B is a Receiver Operator Characteristics (ROC) curve of blood serum concentrations of Apolipoprotein E3 protein (spot N3314) when used to distinguish between two Alzheimer's disease (AD) groups as a function of whether Apolipoprotein E4 protein (spot N5302) is detected (N5302>0) or not detected (N5302=0) in blood serum.

[0029] FIG. **8**A is Dot, Box and Whiskers graph constructed using Analyze-it software for Microsoft Excel, illustrating the differential expression level (PPM) of Transthyretin "Dimer" protein spot N3307 in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P). FIG. **8**B is Receiver Operating Characteristic (ROC) curve of Transthyretin "Dimer" spot N3307 when used as a single biomarker to differentiate between Alzheimer's disease (AD) patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.66±0.023, sensitivity, specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.

[0030] FIG. **9**A is a statistical Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of Transthyretin "Dimer" protein spot N3307 in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from age matched normal controls (AMC), and patients with Alzheimer's disease. FIG. **9**B is a Receiver Operating Characteristic (ROC) curve of Transthyretin (spot N3307) when used to distinguish between patients with Alzheimer's disease (AD) and age-matched control (AMC) subjects as a function of Apolipoprotein E4 spot N5302 when not detected (N5302=0) and when detected (N5302>0) in the 2D gels of their blood serum.

[0031] FIG. **10**A is a statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of Complement Factor H/Hs protein spot N4411 in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P). FIG. **10**B is a Receiver Operating Characteristic (ROC) curve of Complement factor H/Hs protein spot N4411 when used as a single biomarker to differentiate between Alzheimer's disease (AD) patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.59±0.024 sensitivity, specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.

[0032] FIG. **11**A is a statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of Complement Factor H/Hs protein (spot N4411) in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from age matched

normal controls (AMC), and patients with Alzheimer's disease. FIG. **11**B is a Receiver Operating Characteristic (ROC) curve of Complement Factor H/Hs protein (spot N4411) when used to distinguish between Patients with Alzheimer's disease (AD) and age-matched control (AMC) subjects as a function of Apolipoprotein E4 (spot N5302) when not detected (N5302=0) and when detected (N5302>0) in the 2D gels of their blood serum.

[0033] FIG. **12**A is a statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of Complement Factor Bb protein (spot N7616) in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P). FIG. **12**B is a Receiver Operating Characteristic (ROC) curve of Factor Bb protein (spot N7616) when used as a single biomarker to differentiate between Alzheimer's disease (AD) patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.53±0.024 sensitivity, specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.

[0034] FIG. **13**A is a statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of Complement Factor Bb protein (spot N7616) in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from age matched normal controls (AMC), and patients with Alzheimer's disease. FIG. **13**B is a Receiver Operating Characteristic (ROC) curve of Complement Factor Bb protein (spot N7616) when used to distinguish between patients with Alzheimer's disease (AD) and age-matched control (AMC) subjects as a function of Apolipoprotein E4 (spot N5302) when not detected (N5302=0) and when detected (N5302 >0) in the 2D gels of their blood serum.

[0035] FIGS. **14**A-**14**D are statistical Box and Whiskers graphs (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression levels (PPM) of (FIG. **14**A) Complement C3c1 phosphoprotein (spot N7310), (FIG. **14**B) Complement C3dg protein spot N1511, derived from a different amino acid sequence of the C3 parent located just downstream of that shared by C3c1 protein spot N7310, and C3c2a protein spot N9311, (FIG. **14**C) Complement C3c2a protein spot N9311, unphosphorylated form of Complement C3c1, and (FIG. **14**D) the sum of the Complement C3c and C3dg proteins (N7310+N9311+N1511), based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD),with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P) and by Sensitivity, Specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.

[0036] FIGS. **15**A-**15**D illustrate the Receiver Operator Characteristics (ROC) curves (constructed using Analyze-it software for Microsoft Excel), of: (FIG. **15**A) Complement C3c1 phosphoprotein spot N7310, (FIG. **15**B) Complement C3dg protein spot N1511, (derived from a different amino acid sequence of the C3 parent located just downstream of that shared by C3c1 protein spot N7310, and C3c2a protein spot N9311, (FIG. **15**C) Complement C3c2a protein spot N9311, (unphosphorylated form of Complement C3c1), when each is used separately, and (FIG. **15**D) the sum of the Complement C3c and C3dg proteins (N7310+N9311+N1511), to distinguish between patients with Alzheimer's disease (AD) and age-matched control (AMC) subjects.

[0037] FIGS. **16**A-**16**D depicts Dot, Box and Whiskers graphs (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression levels (PPM) of: (FIG. **16**A) Complement C3c1 phosphoprotein spot N7310, (FIG. **16**B) Complement C3dg protein spot N1511, derived from a different amino acid sequence of the C3 parent located just downstream of that shared by C3c1 protein spot N7310 and C3c2a protein spot N9311, (FIG. **16**C) Complement C3c2a protein spot N9311, unphosphorylated form of Complement C3c1, and (FIG. **16**D) the sum of the Complement C3c and C3dg proteins (N7310+N9311+N1511), based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from age matched normal controls (AMC), and patients with Alzheimer's disease as a function of Apolipoprotein E4 protein spot N5302, when it is detected (N5302>0) or not detected (N5302=0) in the 2D gels of their blood serum.

[0038] FIGS. **17**A-**17**D depicts the Receiver Operator Characteristics (ROCs) curves (constructed using Analyze-it software for Microsoft Excel) of (FIG. **17**A) Complement C3dg protein spot N1511, derived from a different amino acid sequence of the C3 parent located just downstream of that shared by C3c1 phosphoprotein spot N7310, and C3c2a protein spot N9311, (FIG. **17**B) Complement C3c1 phosphoprotein spot N7310 in blood serum, (FIG. **17**C) Complement C3c2a protein spot N9311, unphosphorylated form of Complement C3c1, when each is used separately, and (FIG. **17**D) the sum of the Complement C3c and C3dg proteins (N7310+N9311+N1511), to distinguish between patients with Alzheimer's disease (AD) and age matched normal control (AMC) subjects as a function of whether Apolipoprotein E4 protein spot N5302, when it is detected (N5302 >0) or not detected (N5302=0) in blood serum.

[0039] FIGS. **18**A-**18**F depict the linear regression correlation of the blood serum expression level (PPM) of (FIG. **18**A-**18**C) Complement C3c1 phosphoprotein spot N7310, and (FIG. **18**D-18F) Complement C3c2a protein spot N9311, unphosphorylated form of Complement C3c1, with the severity of the Alzheimer's disease, measured clinically by Mini-

Mental State Examination (MMSE) score, when the expression level of Apolipoprotein E4 (N5302) is detected (N5302>0; FIG. **18**B, FIG. **18**E) or not detected (N5302=0; FIG. **18**A, FIG. **18**D) in the blood serum. Severity of Alzheimer's disease increases with decreasing MMSE score (Mild: MMSE=25-20; Moderate: MMSE=19-11; Severe: MMSE≦10). A Box and Whisker graph (FIG. **18**C, FIG. **18**F) illustrate the comparative blood serum expression level (PPM) of Complement C3c1 N7310 in age-matched control (AMC) subjects. Linear regression and Box and Whisker graphs were constructed using Analyze-it software for Microsoft Excel.

[0040] FIGS. **19**A-**19**C are statistical linear regression correlation of the blood serum expression level (PPM) of Complement C3dg protein spot N1511 with the severity of the Alzheimer's disease, measured clinically by Mini-Mental State Examination (MMSE) score, when the expression level of Apolipoprotein E4 protein spot N5302 is detected (N5302>0; (FIG. **19**C) or not detected (N5302=0; FIG. **19**A) in the blood serum. Severity of the Alzheimer's disease increases with decreasing MMSE score (Mild: MMSE=25-20; Moderate: MMSE=19-11; Severe: MMSE 510). A Box and Whisker graph (FIG. **19**B) illustrates the comparative blood serum expression level (PPM) of Complement C3dg protein spot N1511 in age-matched control (AMC) subjects. Linear regression and Box and Whisker graphs were constructed using Analyze-it software for Microsoft Excel.

[0041] FIG. **20** is a summary diagram for the proposed functional relationships between the expression level of Complement protein biomarkers C3c1 protein spot N7310, C3c2a protein spot N9311, and C3dg protein spot N1511, Alzheimer's disease severity, and inflammatory response, when Apolipoprotein E4 N5302 protein was detected (N5302>0) or not detected (N5302=0) in blood serum of Alzheimer's disease patients. The diagram depicts the capacity for early detection of Alzheimer's disease, the measurement of disease severity and of the disease mechanism.

[0042] FIGS. **21**A-**21**D depict statistical Dot, Box and Whiskers graphs (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression level (PPM) of Haptoglobin HP-1 proteins (FIG. **21**A) spot N1514, (FIG. **21**B) spot N2401, (FIG. **21**C) Spot N2407 and (FIG. **21**D) spot N3409, based on the quantitative 2D gel triplicate analysis data, obtained with samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P) calculated by Analyze-it for Microsoft Excel with these data. E) Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel), of biomarker N3409 when used as a single marker to distinguish between AD and PD patients.

[0043] FIG. **22**A depicts a statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the differential expression level (PPM) of the Total of Haptoglobin HP-1 proteins (spots N1514+N2401+N2407+N3409), in blood serum, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including:
Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P) and by Sensitivity, Specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data.
FIG. **22**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Haptoglobin HP-1 protein spots N1514+N2401+N2407+N3409, when used to distinguish between AD patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.59±0.024.

[0044] FIGS. **23**A-**23**D depict statistical Dot, Box and Whiskers graphs (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression levels (PPM) of Haptoglobin HP-1 proteins: (FIG. **23**A) spot N1514, (FIG. **23**B) spot N2401, (FIG. **23**C) Spot N2407, and (FIG. **23**D) spot N3409, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from patients with Alzheimer's disease (AD) and age matched normal controls as a function of whether Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls.

[0045] FIGS. **24**A-**24**D depict the Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Haptoglobin HP-1 proteins: FIG. **24**A) spot N1514, FIG. **24**B) spot N2401, FIG. **24**C) Spot N2407, and FIG. **24**D) spot N3409, when used separately to distinguish between patients with Alzheimer's disease (AD) and age matched normal controls (AMC) as a function of whether Apolipoprotein E4 protein (spot N5302) is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls.

[0046] FIG. **25**A depicts a statistical Dot, Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression level (PPM) of the Total of Haptoglobin HP-1 proteins (spots N1514+N2401+N2407+N3409) as a function of whether Apolipoprotein E4 protein (spot N5302) is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls. FIG. **25**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Haptoglobin HP-1 protein total spots (N1514+N2401+N2407+3409) when used to distinguish between AD patients and age-matched control (AMC) subjects as a function of whether Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected

(N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls.

[0047] FIG. **26**A is a Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression level (PPM) of Inter-alpha-trypsin Inhibitor Heavy Chain (H4) related 35 KD protein (spot N2307), based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P) and by Sensitivity, Specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data. FIG. **26**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Inter-alpha-trypsin Inhibitor Heavy Chain (H4) related 35 KD protein (spot N2307) when used to distinguish between AD patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.62±0.023.

[0048] FIG. **27**A is a statistical Box and Whiskers graph illustrating the blood serum differential expression level (PPM) of Inter-alpha-trypsin Inhibitor Heavy Chain (H4) related 35 KD protein (spot N2307) as a function of whether Apolipoprotein E4 protein (spot N5302) is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls. FIG. **27**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Inter-alpha-trypsin Inhibitor Heavy Chain (H4) related 35 KD protein (spot N2307) as a function of whether Apolipoprotein E4 protein spot N5302 when used to distinguish between AD patients and age-matched control (AMC) subjects as a function of whether Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls.

[0049] FIGS. **28**A-**28**C are statistical linear regression correlation of the blood serum expression level (PPM) of Inter-alpha-trypsin Inhibitor Heavy Chain (H4) related 35 KD protein spot N2307, with the severity of the Alzheimer's disease, measured clinically by Mini-Mental State Examination (MMSE) score, when the expression level of Apolipoprotein E4 protein spot N5302 is detected (N5302>0; FIG. **28**C) or not detected (N5302=0; FIG. **28**A) in the blood serum. Severity of the Alzheimer's disease increases with decreasing MMSE score (Mild: MMSE=25-20; Moderate: MMSE=19-11; Severe: MMSE≦10). Box and Whisker graph (FIG. **28**B) illustrates the comparative blood serum expression level (PPM) of Inter-alpha-trypsin Inhibitor Heavy Chain (H4) related 35 KD protein spot N2307 in age-matched control (AMC) subjects. Linear regression and box and Whisker graphs were constructed using Analyze-it software for Microsoft Excel.

[0050] FIG. **29**A is a statistical Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression level (PPM) of Immunoglobulin Light Chain Protein spot N6224, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P) and by Sensitivity, Specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data. FIG. **29**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Immunoglobulin Light Chain Protein (spot N6224) when used to distinguish between AD patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.64±0.023.

[0051] FIG. **30**A is a statistical Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression level (PPM) of Immunoglobulin Light Chain Protein spot N6224 as a function of whether Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls. FIG. **30**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Immunoglobulin Light Chain Protein spot N6224 as a function of whether Apolipoprotein E4 protein (spot N5302) when used to distinguish between AD patients and age-matched control (AMC) subjects as a function of whether Apolipoprotein E4 protein (spot N5302) is detected (N5302>0) or not detected (N5302=0) in blood serum.

[0052] FIG. **31**A is a statistical Box and Whiskers graph (constructed using Analyze-it software for Microsoft Excel), illustrating the blood serum differential expression level (PPM) of Apolipoprotein A-IV Protein spot N2502, based on the quantitative 2D gel triplicate analysis data obtained with blood serum samples from: 75 normal control individuals (Controls), 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD), with examples of the differences between the ranges of the patient and control groups and their statistical significance by analysis of variance (ANOVA-P) and by Sensitivity, Specificity, and ROC probability (ROC-P), calculated by Analyze-it for Microsoft Excel with these data. FIG. **32**B is a Receiver

Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Apolipoprotein A-IV Protein (spot N2502) when used to distinguish between AD patients and age-matched control (AMC) subjects with an area under the curve (AUC) of 0.64±0.023.

[0053] FIG. **32**A is a statistical Box and Whiskers graph illustrating the blood serum differential expression level (PPM) of Apolipoprotein A-IV Protein spot N2502 as a function of whether Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected (N5302=0) in blood serum of the Alzheimer's disease (AD) patients and the age matched normal controls. FIG. **32**B is a Receiver Operator Characteristics (ROC) curve (constructed using Analyze-it software for Microsoft Excel) of Apolipoprotein A-IV Protein (pot N2502 as a function of whether Apolipoprotein E4 protein spot N5302, when used to distinguish between AD patients and age-matched control (AMC) subjects as a function of whether Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected (N5302=0) in blood serum.

[0054] FIG. **33** illustrates the enhanced sensitivity obtained using the blood serum concentrations of protein biomarkers. The approach employs the separation of Alzheimer's disease patients and age-matched control subjects into two categories, based on the detection or lack of detection of Apolipoprotein E4. N5302 in their blood serum. A multivariate biostatistical analysis is applied to each of the 2 groups, employing all the biomarkers (N3314, N3317, N4411, N7616, HP-1 total [N1514+N2401+N2407+N3409], N7310, N9311, N1511, N2307, N2502, and N6224), followed by summing the separate results of the 2 multivariate biostatistical analyses of the sorted categories. As shown, this approach provides substantial improvement in diagnostic capability (sensitivity increased from 69.6% to 82.3%) over the non-sorted approach, which includes combining all the biomarkers and all the samples into a single multivariate biostatistical analysis.

[0055] FIG. **34** illustrates the 5 types of differences in the differential expression of the protein biomarkers in the blood serum of the sorted Alzheimer's disease patients in relation to the measured concentrations of Apolipoprotein E4 protein spot N5302, when it is detected (N5302>0) and not detected (N5302=0) in the blood. These differences form the basis for the improvements of sensitivity of diagnosis of Alzheimer's disease illustrated in FIG. **33**. In type 1, the serum expression level (PPM) of biomarkers Apolipoprotein E3 protein spot N3314 and Transthyretin dimer protein spot N3307 in Alzheimer's disease patients are lower than age-matched control (AMC) subjects, when Apolipoprotein E4 protein spot N5302 is detected (N5302>0) or not detected (N5302=0) in serum. In type 2, the serum expression level (PPM) of biomarkers Complement Factor H protein spot N4411 and Complement Factor Bb protein spot N7616 in Alzheimer's disease patients are higher than age-matched control (AMC) subjects, when Apolipoprotein E4 protein spot N5302 is not detected (N5302=0), while equal to the serum expression levels of AMC, when N5302 is detected (N5302>0) in serum. In type 3, the serum expression level (PPM) of biomarkers Haptoglobin HP-1 total protein spots N1514+N2401+N2407+N3409 and ITI (H4) RP 37 KD protein spot N2307 in Alzheimer'patients are higher than age-matched control (AMC) subjects, when Apolipoprotein E4 protein spot N5302 is not detected (N5302=0) and equal to serum expression levels of AMC subjects, when N5302 is detected (N5302>0) in serum. In type 4, the serum expression level (PPM) of biomarkers Apolipoprotein A-IV protein spot N2502 and Immunoglobulin light chain protein spot N6224 in Alzheimer's disease patients are lower than age-matched control (AMC) subjects, when Apolipoprotein E4 protein spot N5302 is detected (N5302>0) and not detected (N5302=0) in serum. In type 5, the serum expression level (PPM) of biomarkers Complement C3csum protein spots N7310+N9311+N1511 and Complement Factor I protein spot N 1416 in Alzheimer's disease patients are higher than age-matched control (AMC) subjects, when Apolipoprotein E4 protein spot N5302 is detected (N5302>0) and not detected (N5302=0) in serum

[0056] FIG. **35** illustrates the differences in the disease pathways of neuronal degeneration, and which predominate or are attenuated, based on the differences in the differential expression of the protein biomarkers in the blood serum of the sorted Alzheimer's disease patients as illustrated in FIG. **34**. In patients with Alzheimer's disease, when the serum expression level of Apolipoprotein E4 protein spot N5302 is detected (N5302>0, A and B), the elevated level of this biomarker is associated with A) markedly reduced serum expression of Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein spot N3307, (FIG. **34**, Type 1), enhanced A_plaque and accumulation of Neurofibrillary tangles (NET), and elevated inflammatory cytokines in blood. These changes lead to neuronal oxidative stress and apoptosis and also initiate B) secondary immune and innate inflammatory responses that enhance neuronal degeneration, associated with increased serum levels of phosphorylated C3c1 protein spot N7310, Factor Bb protein spot N7616, non-phosphorylated Complement C3c2a protein spot N9311, C3dg protein spot N1511, and ITI(H4)RP. In patients with Alzheimer's disease, when the serum expression level of Apolipoprotein E4 protein spot N5302 is not detected (N5302=0, C and D), the non-detected level of N5302 is associated with slightly decreased serum expression of Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein spot N3307. The close to normal levels of these 2 biomarkers are associated with neuronal protection. However, these Alzheimer's patients showed elevated serum level of phosphorylated Complement C3c1 protein spot N7310, Factor Bb protein spot N7616, Factor H protein spot N4411, non-phosphorylated Complement C3c2a protein spot N9311, and Complement C3dg protein spot N1511, and ITI(H4)RP protein spot N2307. These biomarkers are associated with autoimmune and innate inflammatory responses, which lead to neuronal degeneration.

[0057] FIGS. **36**A (auto-immune driven) and **36**B (oxidative stress driven) illustrate the differences in Alzheimer's disease biochemical mechanisms of neuronal degeneration, and whether they predominate or are attenuated, based on the identities, the biochemical roles of the protein biomarkers, and the differences in the disease pathways illustrated in FIG. **35**.

[0058] FIG. **37** shows a visual representation of the statistical confidence levels.

[0059] Table 1 depicts the reproducibility of quantitation in 2D gels whereby 9 replicate analyses were performed with an individual sample of bovine serum albumin standard, where the sample was separated by 2D gel electrophoresis into a characteristic set of 5 spots which were then subjected to quantitation. The raw density counts (Gaussian Peak Values) are shown as are the individual values, averages, standard

deviations, % Coefficients of Variation, and the quantity of the protein in nanograms (ng) for each spot.

[0060] Table 2 illustrates the reproducibility of quantitation of protein spots over the dynamic range of the 2D gel assay of human serum depicted in FIG. **1**A. Shown are replicate (14×) 2D gel analyses each of the quantitation of 13 different protein spots ranging from 13,542 ppm to 72 ppm with a coefficient of variation of ≦20% (n=14) where 72 ppm is approximately 10 fold higher than the limit of detection (LOD=5-10 ppm) of the assay.

[0061] Table 3 illustrates the summary statistics for the graph depicted in FIG. **3**.

[0062] Table 4 illustrates the summary statistics for the graph depicted in FIG. **4**.

[0063] Table 5 illustrates the summary statistics for the graph depicted in FIG. **5**.

[0064] Table 6 illustrates the summary statistics for the graph depicted in FIG. **6**.

[0065] Table 7 illustrates the summary statistics for the graph depicted in FIG. **7**.

[0066] Table 8 illustrates the summary statistics for the graphs depicted in FIG. **8**.

[0067] Table 9 illustrates the summary statistics for the graphs depicted in FIG. **9**.

[0068] Table 10 illustrates the summary statistics for the graphs depicted in FIG. **10**.

[0069] Table 11 illustrates the summary statistics for the graph depicted in FIG. **11**.

[0070] Table 12 illustrates the summary statistics for the graph depicted in FIG. **12**.

[0071] Table 13 illustrates the summary statistics for the graph depicted in FIG. **13**.

[0072] Table 14 illustrates the summary statistics for the graph depicted in FIG. **14**.

[0073] Table 15 illustrates the summary statistics for the graphs depicted in FIG. **15**.

[0074] Table 16 illustrates the summary statistics for the graphs depicted in FIG. **16**.

[0075] Table 17 illustrates the summary statistics for the graph depicted in FIG. **17**.

[0076] Table 18 illustrates the summary statistics for the graph depicted in FIG. **22**.

[0077] Table 19 illustrates the summary statistics for the graphs depicted in FIG. **25**.

[0078] Table 20 illustrates the summary statistics for the graph depicted in FIG. **26**.

[0079] Table 21 illustrates the summary statistics for the graph depicted in FIG. **27**.

[0080] Table 22 illustrates the summary statistics for the graph depicted in FIG. **29**.

[0081] Table 23 illustrates the summary statistics for the graph depicted in FIG. **30**.

[0082] Table 24 illustrates the summary statistics for the graph depicted in FIG. **31**.

[0083] Table 25 illustrates the summary statistics for the graphs depicted in FIG. **32**.

[0084] Table 26: illustrates the summary statistics of multivariate linear discriminant analysis (constructed using SAS software) for the graph in FIG. **33**.

[0085] Table 27 illustrates the different disease mechanisms of familial and sporadic neurodegenerative diseases revealed by the patients' blood serum biomarkers

[0086] Table 28 illustrates the different disease mechanisms of PD and ALS neuronal degeneration revealed by patients' blood serum biomarkers.

[0087] Table 29 illustrates the general applications of the invention.

[0088] SEQ ID NO. 1 illustrates the identification of the amino acid sequence of the Apolipoprotein E4 protein precursor of protein spot N5203 wherein amino acids 1-17 are the signal peptide or leader sequence which is removed to make the mature protein.

[0089] SEQ ID NO. 2 illustrates the identification of the amino acid sequence of protein spot N5302 as the full size mature Apolipoprotein E4 protein after trimming the signal peptide off the amino terminal end of the molecule.

[0090] SEQ ID NO. 3 illustrates the identification of the amino acid sequence of the Apolipoprotein E3 protein precursor of protein spot N3314 wherein amino acids 1-17 are the signal peptide or leader sequence which is removed to make the mature protein.

[0091] SEQ ID NO. 4 illustrates the identification of the amino acid sequence of protein spot N3314 as the full size mature Apolipoprotein E3 protein after trimming the signal peptide off the amino terminal end of the molecule.

[0092] SEQ ID NO. 5 illustrates the identification of the amino acid sequence of Transthyretin "Dimer" Protein spot N3307, whose molecular weight by 2D gel electrophoresis is twice that of the molecular weight estimated using the amino acid sequence.

[0093] SEQ ID NO. 6 illustrates the identification of the amino acid sequence of Complement C3, the parent precursor protein of Complement C3c1 protein spot N7310 (Tyrosine Phosphorylated, amino acids 749-951); C3c2a protein spot N9311 (not tyrosine phosphorylated, amino acids 749-951); and C3dg protein spot N1511 (amino acids 955-1303).

[0094] SEQ ID NO. 7 illustrates the identification of the amino acid sequence of tyrosine phosphorylated Complement C3c1 Protein spot N7310, derived from the tyrosine phosphorylated variant of Complement C3 (SEQ ID NO. 6, amino acids 749-951).

[0095] SEQ ID NO. 8 (identical to SEQ ID NO. 7 but not tyrosine phosphorylated) illustrates the identification of the amino acid sequence of Complement C3c2a protein spot N9311, derived from the non tyrosine phosphorylated variant of Complement C3 (SEQ ID NO. 6, amino acids 749-951).

[0096] SEQ ID NO. 9 illustrates the identification of the amino acid sequence of Similar to C3, alternative parent precursor for an alternative C3dg isoform of protein spot N1511 (amino acids 902-1256), but not for C3c1 protein spot N7310 nor for C3c2a protein spot N9311.

[0097] SEQ ID NO. 10 illustrates the identification of the amino acid sequence of Complement C3dg protein spot N1511, derived from Complement C3 (SEQ ID NO. 6, amino acids 955-1303).

[0098] SEQ ID NO. 11 illustrates the identification of the amino acid sequence of Complement C3dg alternate isoform for protein spot N1511, derived from Similar to C3 (amino acid SEQ ID NO. 9; amino acids 902-1256).

[0099] SEQ ID NO. 12 illustrates the identification of the amino acid sequence of Complement Factor Bb protein spot N7616.

[0100] SEQ ID NO. 13 illustrates the identification of the amino acid sequence of Complement Factor H Parent Protein precursor of Complement Factor H/Hs protein spot N4411.

[0101] SEQ ID NO. 14 illustrates the identification of the amino acid sequence of Complement Factor Hs (Short Splice Form) alternate parent of Complement Factor H/Hs protein spot N4411.

[0102] SEQ ID NO. 15 illustrates the amino acid sequence of Complement Factor H/Hs protein spot N4411, derived from either SEQ ID NO. 13 and/or SEQ ID NO. 14.

[0103] SEQ ID NO. 16 illustrates the identification of the amino acid sequence of Inter alpha trypsin inhibitor heavy (H4) chain related protein, parent of the 35 KD protein spot N2307.

[0104] SEQ ID NO. 17 illustrates the identification of the amino acid sequence of Inter alpha trypsin inhibitor heavy (H4) chain related 35 KD protein isoform 1, protein spot N2307.

[0105] SEQ ID NO. 18 illustrates the identification of the amino acid sequence of Inter alpha trypsin inhibitor heavy (H4) chain related protein 35 KD isoform 2, alternate protein of spot N2307.

[0106] SEQ ID NO. 19 illustrates the identification of the amino acid sequence of Haptoglobin HP-1 Protein spots N1514; N2401; N2407; N3409.

[0107] SEQ ID NO. 20 illustrates the identification of the amino acid sequence of Complement Factor I Protein spot N1416.

[0108] SEQ ID NO. 21 illustrates the identification of the amino acid sequence of Immunoglobulin Light Chain Protein spot N6224.

[0109] SEQ ID NO. 22 illustrates the identification of the amino acid sequence of Apolipoprotein A-IV Protein spot N2502.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0110] The present invention relates to protein biomarkers for Alzheimer's disease, whereby lack of detection, detection, and/or the quantity of a first protein biomarker in a biological sample from Alzheimer's disease patients correlates with significant differences in the quantities of other protein biomarkers of Alzheimer's disease. When Alzheimer's disease patients and age-matched normal control subjects are each placed into separate categories based on whether they do or do not have detectable quantities of the first protein biomarker, the protein identities of, and the differences in the quantities of the first protein biomarker and/or one or more other protein biomarkers in the biological sample provide opportunities: to improve sensitivity and specificity of differential diagnosis. To measure disease severity and monitor drug response. To monitor drug clinical trial stratification of patients. To indicate differences in neuronal degeneration mechanisms in the patients. To measure the activity of these mechanisms. To determine which of these mechanisms predominates. To determine which biomarkers and mechanisms measure the severity of Alzheimer's disease in the patients. To discover new targeted therapies. To develop companion diagnostics.

[0111] More particularly, a preferred embodiment of the present invention relates to the identification of the relationships between two or more of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H/Hs protein, a Complement Factor I protein, an Immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein, as biomarkers for distinguishing between different categories or types of Alzheimer's disease, and for early detection, screening, diagnosis, differential diagnosis, and monitoring of disease severity and disease mechanisms of patients with Alzheimer's disease (AD), Alzheimer's disease Like (AD-Like) dementias, Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease), and Parkinson's disease. In this embodiment, the lack of detection, detection, and/or the quantity of the first protein biomarker, an Apolipoprotein E4 protein, and the quantities of one or more of an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H/Hs protein, a Complement Factor I protein, an immunoglobulin protein, a Haptoglobin protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein, are employed for distinguishing between different categories or types of Alzheimer's disease, and for early detection, screening, diagnosis, differential diagnosis, and monitoring of disease severity and disease mechanisms of patients with Alzheimer's disease (AD), Alzheimer's disease like (AD-Like) dementias, Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease), and Parkinson's disease (PD).

[0112] The method for identification of an Apolipoprotein E4 protein as a biomarker for Alzheimer's disease is based on the comparison of 2D gel electrophoretic images of serum obtained from human subjects with and without diagnosed Alzheimer's disease.

[0113] 2D gel electrophoresis has been used in research laboratories for biomarker discovery since the 1970's (7-16). In the past, this method has been considered highly specialized, labor intensive and non-reproducible. Only recently with the advent of integrated supplies, robotics, and software, combined with bioinformatics, has progression of this proteomics technique in the direction of diagnostics become feasible. The promise and utility of 2D gel electrophoresis is based on its ability to detect changes in expression of intact proteins and to separate and discriminate between specific intact protein isoforms that arise due to variations in amino acid sequence and/or post-synthetic protein modifications such as phosphorylation, ubiquitination, conjugation with ubiquitin-like proteins, acetylation, glycosylation, and proteolytic processing. These are critical features in cell regulatory processes that are differentially expressed in blood serum biomarkers in neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, and ALS (Goldknopf, I. L. et al. U.S. Utility patent application Ser. No. 11/507,337, and 17-19).

[0114] There are few comparable alternatives to 2D gel electrophoresis for tracking changes in intact protein expression patterns related to disease. Furthermore, the introduction of high sensitivity fluorescent staining for ultra high sensitivity visualization of characteristic, recognizable protein spot patterns, digital image processing, and computerized quantitative image analysis has greatly amplified and simplified the detection of unique species and the quantification of proteins. By using known protein standards as landmarks within each gel run, computerized analysis can detect unique differences in protein expression and modifications between two samples from the same individual or between several individuals.

[0115] Separated intact protein spots in the 2D gels that of interest can be excised from the gels and the proteins can then be identified by in-gel proteolytic digestion followed by mass spectrometric analysis. This includes matrix assisted laser desorption time of flight mass spectroscopy (MALDI-TOF MS) based peptide mass fingerprinting and database searching, and/or liquid chromatography with tandem mass spectrometry (LC MS/MS) to provide partial sequencing of individual peptides to confirm identification of the proteins

[0116] The identification of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, an Apolipoprotein A-IV Protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H/Hs protein, a Complement Factor 1 protein, a Haptoglobin protein, an immunoglobulin protein, and an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related 35 KD protein as biomarkers of neurodegenerative disease was based on a quantitative comparison of the 2D gel electrophoretic images of blood serum samples obtained from 75 normal/Controls, 115 Alzheimer's disease patients (AD), 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed dementias including Frontotemporal dementia (FTD); Lewy body dementia (LBD); Corticalbasal Ganglionic degeneration (CBGD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA.

Sample Collection and Preparation

[0117] Sample collection and storage have been performed in many different ways depending on the type of sample and the conditions of the collection process. In the present study, serum samples were collected, aliquoted and stored in a −80° C. freezer before analysis.

[0118] In a preferred embodiment of the invention, the serum samples were removed from −80° C. and placed on ice for thawing. To each 100 μL of sample, 100 μL of LB-2 buffer (7M urea. 2M Thiourea, 1% DTT, 1% Triton X-100, 1× Protease inhibitors, and 0.5% Ampholyte pH 3-10) was added and the mixture vortexed. The sample was incubated at room temperature for about 5 minutes.

Two Dimensional Gel Electrophoresis of Samples

[0119] Separation of the proteins in the serum samples was then performed using 2D gel electrophoresis. The 2D gel electrophoretic images were obtained, compared and analyzed as described in the U.S. Utility patent application Ser. No. 11/411,659 filed Apr. 26, 2006 and entitled "Assay for Neuromuscular Diseases" by inventors Goldknopf I L, et al., and as described in the U.S. Utility patent application Ser. No. 11/4487,715 filed Jul. 17, 2006 and entitled "Assay for ALS and ALS-like Disorders" by inventors Goldknopf IL, et al., and as described in the U.S. Utility patent application Ser. No. 11/503,881 filed Aug. 14, 2006 and entitled "Assay for Differentiating Alzheimer's and Alzheimer's-like Disorders" by inventors Goldknopf I L, et al., incorporated herein by reference. A protein assay was performed on the sample to determine total protein content in μg.

[0120] Based on the total protein content in the sample, an aliquot of approximately 100 μg of the protein was suspended in a total volume of 184 μL of IEF loading buffer containing 1 μL Bromophenol Blue as a marker to trace the progress of the electrophoresis. Each sample was loaded onto an 11 cm IEF strip (Bio-Rad), pH 5-8, and overlaid with 1.5-3.0 ml of mineral oil to minimize the sample buffer evaporation. Using the PROTEAN® IEF Cell, an active rehydration was performed at 50V and 20° C. for 12-18 hours.

[0121] IEF strips were then transferred to a new tray and focused for 20 min. at 250V followed by a linear voltage increase to 8000V over 2.5 hours. A final rapid focusing was performed at 8000V until 20,000 volt-hours were achieved. Running the IEF strip at 500V until the strips were removed finished the isoelectric focusing process.

[0122] Isoelectric focused strips were incubated on an orbital shaker for 15 mm with equilibration buffer (2.5 ml buffer/strip). The equilibration buffer contained 6M urea, 2% SDS, 0.375M HCl, and 20% glycerol, as well as freshly added DTT to a final concentration of 30 mg/ml. An additional 15 mm incubation of the IEF strips in the equilibration buffer was performed as before, except freshly added iodoacetamide (C_2H_4INO) was added to a final concentration of 40 mg/ml. The IPG strips were then removed from the tray using clean forceps and washed five times in a graduated cylinder containing the Bio Rad running buffer 1× Tris-Glycine-SDS.

[0123] The washed IEF strips were then laid on the surface of Bio Rad pre-cast CRITERION SDS-gels 8-16%. The IEF strips were fixed in place on the gels by applying a low melting agarose. A second dimensional separation was applied at 200V for about one hour. After electrophoresis, the gels were carefully removed and placed in a clean tray and washed twice for 20 minutes in 100 ml of pre-staining solution containing 10% methanol and 7% acetic acid.

Staining and Analysis of the 2D Gels

[0124] The gels were stained with SyproRuby™ (Bio-Rad Laboratories) fluorescent protein stain and subjected to fluorescent digital image analysis in an FX Imager (Bio-Rad Laboratories). The protein patterns of the serum samples were analyzed using PDQUEST™ (Bio-Rad Laboratories) image analysis software.

[0125] The 2D gel patterns of the 75 serum samples collected from normal control subjects were compared with each other pursuant to the methodology described in the U.S. Utility patent application Ser. No. 11/411,659 filed Apr. 26, 2006 and entitled "Assay for Neuromuscular Diseases" by inventors Goldknopf I L, et al., and as described in the U.S. Utility patent application Ser. No. 11/4487,715 filed Jul. 17, 2006 and entitled "Assay for ALS and ALS-like Disorders" by inventors Goldknopf I L, et al., and as described in the U.S. Utility patent application Ser. No. 11/503,881 filed Aug. 14, 2006 and entitled "Assay for Differentiating Alzheimer's and Alzheimer's-like Disorders" by inventors Goldknopf I L, et al., incorporated herein by reference. The 75 normal individual blood serum samples all gave similar 2D gel protein patterns.

[0126] These normal protein expression patterns were then compared to the gel patterns obtained with blood serum samples from the 115 Alzheimer's disease (AD) patients, 12 Parkinson's disease patients (PD), and 12 patients with AD-Like and Mixed dementias including: Frontotemporal

dementia (FTD); Lewy body dementia (LBD); Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA. When the gel patterns of AD patients were compared to the gel patterns of normal subjects, protein spots N5302, N3314, N3307, N7310, N9311, N1511, N7616, N4411, N 1416, N1514, N2401, N2407, N3409, N6224, N2502, and N2307, of particular interest, were identified as shown in FIG. **2**, and selected for further investigation. Protein spots N5302, N3314, N3307, N7310, N9311, N1511, N7616, N4411, N1416, N1514, N2401, N2407, N3409, N6224, N2502, and N2307 were quantitated by stain intensity in each of the normal and disease patient groups of serum samples.

[0127] In order to assess the reproducibility of the 2D gels and staining, 75 nanograms of bovine serum albumin (BSA) was run on 9 separate 2D gels. The gels were stained with SYPRO RUBY and the 5 spots resolved in the BSA region of the gel were then subjected to quantitative analysis using PDQUEST™ and the Gaussian Peak Value method. The results shown in Table 1 illustrate that the electrophoretic patterns were reproducible and the reproducibility (% Coefficient of Variation=% CV) was independent of the spot amount over the range tested (2.9-38.6 ng/spot).

TABLE 1

Reproducible Quantitation of Bovine Serum Albumin (BSA) Standard (n = 9)

	Spot #				
Replicate #	9901	9902	9904	9905	9906
1	332	1152	2612	739	229
2	246	974	2694	513	167
3	336	1065	2354	668	225
4	311	1272	3482	713	198
5	351	1168	2724	733	245
6	268	1059	2753	622	184
7	452	1630	4000	946	281
8	405	1195	2752	870	274
9	258	1050	2716	699	189
AVG	329	1174	2899	723	221
STDEV	68	193	510	127	40
% CV	21%	16%	18%	18%	18%
ng/spot	4.4	15.6	38.6	9.6	2.9

Reproducibility of Quantitation in 9 Gels—PDQuest Gaussian Peak Value of the Major Components of BSA

[0128] As shown in FIG. **1**A, 2D gel electrophoresis of human blood serum, fluorescent staining with SyproRuby, and digital imaging provides a broad dynamic concentration range of protein spots, which are illustrated by the indicated spots with concentrations ranging from a low of 55 ppm spot density to a high of 15,789 ppm spot density (white arrows). Triplicate analysis with the same blood serum sample shows good reproducibility, with a coefficient of variation=13.8% for the triplicate analysis of the indicated spot (FIG. **1**B). Table 2 illustrates the reproducibility of quantitation of 13 different spots from 2D gel electrophoresis of human blood serum, with decreasing concentrations over the full dynamic range of the assay, illustrated with protein spots ranging in spot density from a low of 72 ppm to a high of 13,542 ppm, with a coefficients of variation ≦20% for replicates of 14 gels run on different days with different technicians, independent of the concentrations of the protein spots within that range. The limit of detection (LOD) is at a 10 fold lower concentration than the bottom of that range, or 100 pg/spot~5-10 PPM

TABLE 2

Reproducible Quantitation of 13 Different Protein Spots (n = 14, Range 72 ppm-13,542 ppm)

Biomarker	N	Mean	+/–	Std Error	Coefficient of Variation	≦20%
M1	14	13542		711	20		
M2	14	3853		140	14		
M3	14	14⍰3		52	14		
M4	14	10⍰5		49	18		
M5	14	678		28	15		
M6	14	655		33	19		
M7	14	595		31	19		
M8	14	469		26	20		
M9	14	359		16	17		
M10	14	209		11	20		
M11	14	129		5	15		
M12	14	106		6	20		
M13	14	72		4	19	⇓

LOD = 100 pg/spot = ~5-10 ppm

⍰ indicates text missing or illegible when filed

The Isolation and Identification of the Protein Spots

[0129] Protein spots N5302 N3314, N3307, N7616, N4411, N1416, N7310, N9311, N1511, N1514, N2401, N2407, N3409, N6224, N2502, and N2307, were carefully excised, in-gel digested with trypsin, and subjected to mass fingerprinting/sequence analysis by high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) and expert database searching.

[0130] Tandem mass spectrometry provides a powerful means of determining the structure and identity of proteins and peptides. The unknown tryptic peptide is first separated and purified by liquid chromatography and then the effluent from the separation is vaporized by electrospray, separated in a mass spectrometer and then bombarded with high-energy electrons causing it to fragment in a characteristic manner, indicative of its amino acid sequence. The fragments, which are of varying mass and charge, are then passed through a magnetic field and separated according to their mass/charge ratios. The resulting characteristic fragmentation pattern of the unknown peptide is used to identify its amino acid sequence.

[0131] A protein can often be unambiguously identified by an LC MS/MS analysis of its constituent peptides (produced by either chemical or enzymatic treatment of the sample).

[0132] Following differential expression analysis, protein spots N5302 N3314, N3307, N7616, N4411, N1416, N7310, N9311, N1511, N1514, N2401, N2407, N3409, N6224, N2502, and N2307, were carefully excised from the gel for identification. Excised gel spots of proteins N5302 N3314, N3307, N7616, N4411, N1416, N7310. N9311, N1511, N1514, N2401, N2407, N3409, N6224 N2502, and N2307, were de-stained by washing the gel spots twice in 100 mM

NH_4HCO_3 buffer, followed by soaking the gel spots in 100% acetonitrile for 10 minutes. The acetonitrile was aspirated before adding the trypsin solution. Typically, a small volume of trypsin solution (approximately 5-15 μg/ml trypsin is added to the de-stained gel spots and incubated at 3 hours at 37° C. or overnight at 30° C. The digested peptides were extracted, washed, desalted and subjected to liquid chromatography followed by tandem mass spectral analysis to identify the protein spots.

[0133] Tandem mass spectrometry of tryptic peptides provides a powerful means of determining the structure and identity of proteins. The unknown tryptic peptides from the digestion are extracted from the gel and first separated and purified by liquid chromatography and then the effluent from the separation is vaporized by electrospray, separated in a mass spectrometer and then bombarded with high-energy electrons causing the peptides to fragment it in a characteristic manner, indicative of their amino acid sequences. The fragments, which are of varying mass and charge, are then passed through a magnetic field and separated according to their mass/charge ratios. The resulting characteristic fragmentation patterns of the unknown peptides are used to identify the amino acid sequence of the protein spot from which they were obtained. Those of skill in the art are familiar with mass spectral analysis of digested peptides. The mass spectral analysis was conducted on a Micromass LC QTOF (Waters). Peptide fragmentation patterns were obtained from the tryptic in-gel digests of the protein spots and the patterns were subjected to public database searches using the GenBank and dbEST databases maintained by the National Center for Biotechnology Information (hereinafter referred to as the NCBI database). Those of skill in the art are familiar with searching databases, like the NCBI database. The NCBI database search results were displayed with the best matched amino acid sequences of the identified peptides and the protein accession of number the protein sequence they were derived from. Biomarkers identified by LC-MS/MS of the in-gel tryptic peptide digests are listed.

[0134] The NCBI database search results were displayed with the best matched amino acid sequences of the identified tryptic peptides and the protein accession numbers of the proteins sequences they were derived from. For protein spots N5302 N3314, N3307, N7616, N4411, N1416, N7310, N9311, N1511, N1514, N2401, N2407, N3409, N6224, N2502, and N2307, the proteins identified by the NCBI database search were: N5302, is an Apolipoprotein E4 protein (Precursor SEQ ID NO. 1, N5302 SEQ ID NO. 2); N3314, an Apolipoprotein E3 (Precursor SEQ ID NO. 3, N3314 SEQ ID NO. 4); N3307, a Transthyretin "Dimer" protein (N3307 SEQ ID NO. 5); 3 Complement C3 proteins; N7310, a Complement C3c1 protein (Precursor SEQ ID NO. 6, N7310 SEQ ID NO. 7); N9311, a Complement C3c2a protein (Precursor SEQ ID NO. 6, N9311 SEQ ID NO. 8,); and N1511, a Complement C3dg protein (Precursor SEQ ID NO. 6, N1511 SEQ ID NO. 10, alternate precursor SEQ ID NO. 9, N1511 alternate SEQ ID NO. 11); N7616, a Complement Factor Bb protein (N7616 SEQ ID NO. 12); N4411, a Complement Factor H/Hs protein (Precursor SEQ ID NO. 13, alternate precursor SEQ ID NO. 14, N4411 SEQ ID NO. 15); and N2307, An Inter-alpha Trypsin Inhibitor protein (Heavy Chain H4 Related Precursor Protein SEQ ID NO. 16, N2307 Heavy Chain H4 isoform 1 SEQ ID NO. 17, N2307 Heavy Chain H4 alternate isoform 2 SEQ ID NO. 18); Four Haptoglobin proteins; N1514 N2401, N2407, and N3409, electrophoretic variants of a Haptoglobin HP-1 protein (N1514 N2401, N2407, N3409 SEQ ID NO. 19); N1416, Complement Factor I protein (N1416 SEQ ID NO. 20); N6224, an Immunoglobulin Light Chain protein (N6224 SEQ ID NO. 21); and N2502, an Apolipoprotein A-IV protein (N2502 SEQ ID NO. 22).

Biostatistical Analysis

[0135] Statistical significance of differences in individual biomarker blood serum concentrations between different patient and control groups is performed using methods well known in the art, Dot Box and Whiskers plots, analysis of variance, and Receiver Operator Characteristics, employing a standard off the shelf software package, "Analyze-it" in Microsoft XL. Box and Whisker plots give a visual representation of non-parametric descriptive statistics. The central "box" (FIG. **37**) represents the distance between the first and third quartiles (inter quartile range or IQR), with the median marked as the horizontal line inside the box. The notch in the box represent the 95^{th} % confidence interval around the median (the 50th percentile); thus groups that display non-overlapping notches can be considered statistically different ($p<0.05$). The minimum value is the origin of the leading "whisker" and the maximum value is the limit of the trailing "whisker". All values are plotted individually (Dots) and those values outside the whiskers are considered possible outliers, presented either as circle (far outlier) or plus sign (near outliers).

Receiver Operating Characteristic (ROC) Curve

[0136] The diagnostic performance of a test or the accuracy of a test to discriminate diseased cases from normal cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. ROC curves can also be used to compare the diagnostic performance of two or more laboratory or diagnostic tests. In ROC curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (1—Specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test (64).

Differential Expression of Protein spots N5302, N3314, N3307, N7616, N4411, N1416, N7310, N9311, N1511, N1514, N2401, N2407, N3409, N6224, N2502, and N2307 in Age Matched Normal Control Subjects, and Patients Diagnosed with Alzheimer's Disease, with Parkinson's disease, and with AD-Like, and/or Mixed Disorders

[0137] The blood serum concentrations of Apolipoprotein E4 protein spot N5302 (FIG. **3**, Table 3), Apolipoprotein E3 protein spot N3314 (FIG. **5**, Table 5), Transthyretin "Dimer" protein N3307 (FIG. **8**, Table 8), Complement Factor H/Hs protein spot N4411 (FIG. **10**, Table 10), Complement Factor Bb protein spot N7616 (FIG. **12**, Table 12), Complement C3c1 protein spot N7310 (FIG. **14**, Table 14), Complement C3c2a protein spot N9311 (FIG. **14**, Table 14), Complement C3dg protein spot N1511 (FIG. **14**, Table 14), C3Sum=N7310+N9311+N1511 (FIG. **14**, Table 14), Haptoglobin HP-1 proteins N1514, N2401, N2407, and N3409 (FIGS. **23**, **24**), Total Haptoglobin HP-1=N1514+N2401+N2407+N3409 (FIG. **25**, Table 19), Inter-alpha Trypsin

Inhibitor Heavy Chain H4 related 35 KD protein N2307 (FIG. **26**, Table 20), Immunoglobulin Light Chain Protein N6224 (FIG. **29**, Table 22), and Apolipoprotein A-IV protein N2502 (FIG. **31**, Table 24) were all determined by triplicate 2D gel analysis of individual blood serum samples from 75 age matched normal controls, 115 Alzheimer's disease (AD) patients, 12 Parkinson's disease patients (PD), and 12 patients with AD-Like or Mixed dementias, 12 patients with AD-Like and Mixed disorders, including: Frontotemporal dementia (FTD), Frontotemporal dementia combined with Chronic Inflammatory Demyelinating polyneuropathy, Lewy body dementia (LBD), Vascular (Multi-infarct) dementia, Thalamic CVA combined with HX or Lung CA, Post-irradiation Encephalopathy, Seizures, Alcohol related dementia, Semantic dementia, Memory Dysfunction, neuro exam "Normal," and Corticalbasal Ganglionic Degeneration (CBGD).

Apolipoprotein E4 and Apolipoprotein E3

[0138] Shown in FIG. **3** and Table 3 are the differences in the blood serum concentration of Apolipoprotein E4 protein N5302 in AD, PD and ADL (Alzheimer-like disorders) as percent difference from age matched normal controls (AMC). The application of Apolipoprotein E4 (spot N5302) as a single biomarker to differentiate between Alzheimer's disease patients and age-matched control (AMC) subjects shows more trend towards specificity (74.7%).

[0139] The Apolipoprotein E4 protein N5302 is the protein product of the Apo E ϵ4 gene allele. The Apo E ϵ4 gene allele is known to be associated with increased risk of dementia, and is inherited as one of three Apo E gene alleles, termed ϵ2, ϵ3, and ϵ4, with mean frequencies in the general population of about 8%, 78%, and 14%, respectively (3). The degree of risk of dementia conferred by Apo E ϵ4 allele rises in a "gene dose" dependent manner (4), increasing with the number of Apo E ϵ4 alleles inherited, from: ϵ4 non-carriers; to ϵ4/ϵ3 and ϵ4/ϵ2 hetero-zygotes; to ϵ4/ϵ4 homo-zygotes (5), all capable of developing Alzheimer's disease, although those lacking Apo E ϵ4 allele have the least risk of developing AD, and also may tend to get the disease at a later age of onset (6). In a preferred embodiment of the invention, those Alzheimer's disease patients and age matched normal controls who have detectable levels of Apolipoprotein E4 protein in their blood serum (N5302>0) are assumed to be either Apo E ϵ4/ϵ3 or ϵ4/ϵ2 hetero-zygotes, or ϵ4/ϵ4homo-zygotes, and to not be Apo E ϵ4 non-carriers. Also in a preferred embodiment of the invention those Alzheimer's disease patients and age matched normal controls who have no detectable levels of Apolipoprotein E4 protein in their blood serum (N5302=0) are assumed to be Apo E ϵ4 non-carriers, although there may be some individuals in this group who have the Apo E ϵ4 allele in their genome but it is unexpressed as protein or expressed below the level of detection of the 2D gel electrophoresis method employed.

[0140] In the preferred embodiment of the invention, the detection, or a lack of detection of Apolipoprotein E4 protein N5302 expression, as measured in blood serum, whether Apolipoprotein E4 protein concentration is detected (N5302>0), or is not detected (N5302=0), is determined and its effect upon the expression of other blood serum biomarkers of Alzheimer's disease, measured as changes in blood serum concentration, are used to measure differences in the form that Alzheimer's disease takes in the patient.

[0141] As shown in FIGS. **3**A and **4**A, and accompanying Tables 3 and 4, AD patients have significantly higher blood serum concentrations of Apolipoprotein E4 protein spot N5302 than age matched normal controls (ANOVA-P<0.0001), Parkinson's disease patients, and patients with Alzheimer's disease-like dementias. In the case of Alzheimer's disease (AD) vs. age matched normal controls (AMC), using a cutoff of N5302>0; the separation between the AD and AMC groups is less sensitive for detection of Alzheimer's disease (FIG. **3***b*, Table 3, Receiver Operator Characteristics, ROC Sensitivity=55.1%, ROC-P<0.0001) and more specific for detection of age matched normal controls (FIG. **3***b*, Table 3, ROC Specificity=74.7%, ROC-P<0.0001), reflecting the increased risk of Alzheimer's disease in those who have the Apo E ϵ4 allele and who express the allele as protein in blood serum (Apolipoprotein E4 protein spot N5302>0). Moreover, in AD and AMC individuals who have detectable levels of Apolipoprotein E4 protein spot N5302 in their blood serum (N5302>0), the level of Apolipoprotein E4 protein spot N5302 is significantly higher in Alzheimer disease patients than in the age matched controls (FIG. **4***a*, N5302>0, ANOVA-P<0.0001). When Apolipoprotein E4 protein N5302 is detected in blood serum (N5302>0), the separation between the AD and AMC groups is sensitive for detection of Alzheimer's disease (FIG. **4**B, Table 4, ROC Sensitivity=64.2%, ROC-P<0.0030) but not specific for age matched normal controls ((FIG. **4**B, Table 4, ROC Specificity=50.7%, ROC-P<0.0030). This indicates that in addition to its detection or lack of detection, the level of expression of Apolipoprotein E4 protein spot N5302 is also a significant factor, in that an increased level of Apolipoprotein E4 protein spot N5302 demonstrates significant sensitivity for detection of Alzheimer's disease. Also, the reduced specificity reflects increased risk and/or undiagnosed Alzheimer's disease in the age matched normal controls who express the Apo E ϵ4 allele product Apolipoprotein E4 protein N5302 in blood serum (N5302>0).

[0142] As shown in FIG. **5**A and Table 5, age matched normal control subjects have the highest blood serum concentrations of the Apo E ϵ3 allele protein product, Apolipoprotein E3 protein spot N3314. Alzheimer's disease patients, patients with AD-Like and Parkinson's disease patients have significantly lower concentrations of Apolipoprotein ϵ3 protein spot N3314 (ANOVA-P<0.0001), than age-matched normal control (AMC). The reduced level of Apolipoprotein E3 protein spot N3314 in AD is equally sensitive for detection of Alzheimer's disease and specific for age matched normal controls (FIG. **5**B, Table 5, ROC Sensitivity=64.1%, ROC Specificity=64.0, ROC-P<0.0001).

[0143] However, as shown in FIG. **6**A and Table 6, when Alzheimer's disease patients and age matched normal controls are compared on the basis of whether or not Apolipoprotein E4 protein is detected in blood serum (N5302>0 vs. N5302=0, respectively), the Alzheimer's disease patients with detectable blood serum levels of Apolipoprotein E4 protein (N5302>0) have significantly lower expression of Apolipoprotein E3 protein N3314 in blood serum than the Alzheimer's disease patients with no detectable blood serum levels of Apolipoprotein E4 protein (FIG. **6***a* N5302=0).

[0144] When the potential utility for diagnosis of Alzheimer's disease is measured by plotting Receiver Operator Characteristics of blood serum concentrations of Apolipoprotein E3 protein N3314 as a function of whether Apolipoprotein E4 protein N5302 is detected (N5302>0) or not detected (N5302=0) in blood serum (FIG. **7**A, Table 7a, b), it is readily apparent that when Apolipoprotein E4 protein spot N5302

was detected (N5302>0) in the blood serum, the distinguishing of Alzheimer's disease patients from age matched normal controls on the basis of reduced blood serum concentration of Apolipoprotein E3 protein spot N3314 was accomplished with significant sensitivity and specificity (Table 7b, Sensitivity=68.2%, Specificity=68.1%, ROC-P<0.0001; AUC=0.76±0.033). Conversely, when Apolipoprotein E4 protein spot N5302 was not detected (N5302=0) in the blood serum, significantly less sensitivity and specificity was obtained by measuring the concentration of Apolipoprotein E3 spot N3314 (Table 7a, Sensitivity=54.2% Specificity=53.8% ROC-P<0.0004; AUC=0.60±0.033).

[0145] Thus, in active Alzheimer's disease, decreased expression of Apo E ϵ3 wild type allele gene product, the Apolipoprotein E3 protein spot N3314, in blood serum has clinical diagnostic utility, when the detection or lack of detection in blood serum of the Alzheimer's disease risk gene allele Apo E ϵ4 protein product, Apolipoprotein E4 protein spot N5302 is also taken into account.

[0146] Results similar to that obtained for Apolipoprotein E3 protein spot N3314 were also obtained for Transthyretin "Dimer" protein spot N3307 (see FIG. **8**, Table 8; and FIG. **9**, Table 9).

[0147] In a preferred embodiment of the invention, the lack of detection or the detection, and the quantity of Apolipoprotein E4 protein spot N5302, is employed combined with the concentrations of Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein N3307 in blood serum wherein: Concentrations of Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein spot N3307 in blood serum that are significantly below the ranges of age matched normal controls, with detection of Apolipoprotein E4 protein spot N5302, and with concentrations of Apolipoprotein E4 protein spot N5302 significantly above the range of age matched normal controls, are indicated for sensitive and specific detection of Alzheimer's disease.

[0148] For the purposes of the preferred embodiment of this invention, the known association of Apolipoprotein E protein and Transthyretin protein into neurofibrillary tangles and senile plaques, as well as the neuroprotective role of Apolipoprotein E3 against oxidative stress and related signals for apoptosis, indicate significant differences in the mechanisms of neuronal degeneration between these two forms of Alzheimer's disease (wherein either N5302=0 or N5302>0, FIGS. **34-36**).

Complement Factor Bb Protein and Complement Factor H/Hs Protein

[0149] Also in a preferred embodiment of the invention, a lack of detection N5302=0), or the detection (N5302>0) and the quantity of Apolipoprotein E4 protein N5302 as measured in blood serum, is determined and its effect upon the expression of Complement Factor H/Hs protein N4411 and Complement Factor Bb protein N7616 is also determined.

[0150] Complement Factor H/Hs protein N4411 is significantly up-regulated in the blood serum of patients with Alzheimer's disease and Parkinson's disease, but not in patients with AD-like and Mixed dementias, as compared to age matched normal controls (AMC) (FIG. **10**A, Table 10, ANOVA-P<0.0040). Complement Factor Bb protein N7616 is also up-regulated in the blood serum of patients with Alzheimer's disease and Parkinson's disease, and in patients with AD-like and Mixed dementias as well, when compared to age matched normal controls, but the up-regulation in Alzheimer's disease lacks statistical significance (FIG. **12**A, Table 12, ANOVA-P>0.110).

[0151] In the case of Alzheimer's disease (AD) vs. age matched normal controls (AMC), using a cutoff for N4411 of AD>261 ppm, the separation between AD and AMC groups is equally sensitive and specific (FIG. **10**B, Table 10, Sensitivity=57.4%, Specificity=57.3%, ROC-P<0.0002), whereas using a cutoff value of AD>233ppm, Complement Factor Bb protein N7616 demonstrated significantly less sensitivity and specificity (FIG. **12**B, Table 12, Sensitivity=52.5%, Sensitivity=52.4%, ROC-P>0.09).

[0152] However, when Alzheimer's disease patients and age matched normal controls are compared on the basis of whether or not Apolipoprotein E4 protein spot N5302 is detected in blood serum (N5302>0 vs. N5302=0, respectively), an opposite effect to that on Apolipoprotein E3 protein spot N3314, and Transthyretin "Dimer" protein N3307 was seen. The Alzheimer's disease patients without detectable blood serum levels of Apolipoprotein E4 (N5302=0) had significantly higher expression of both Complement Factor H/Hs protein spot N4411 (FIG. **11**A, Table 11, ANOVA-P<0.0001) and Complement Factor Bb protein spot N7616 (FIG. **13**A, Table 13, ANOVA-P<0.0006) in blood serum than age matched normal controls. Alzheimer's disease patients with detectable blood serum levels of Apolipoprotein E4 protein (N5302>0) did not have significantly different levels of expression of either Complement Factor H/Hs protein spot N4411 (FIG. **11**A, Table 11, ANOVA-P>0.80) nor of Complement Factor Bb protein spot N7616 (FIG. **12**A, Table 12, ANOVA-P>0.17) in blood serum than age matched normal controls.

[0153] When the potential utility for diagnosis of Alzheimer's disease is measured by plotting Receiver Operator Characteristics of blood serum concentrations of Complement Factor H/Hs protein spot N4411 (FIG. **11**B, Table 11) and Complement Factor Bb protein spot N7616 (FIG. **13**B, Table 13) as a function of whether Apolipoprotein E4 protein spot N5302 is detected in blood serum, it was found that when Apolipoprotein E4 protein spot N5302 was not detected (N5302=0) in the blood serum, the distinguishing of Alzheimer's disease patients from age matched normal controls on the basis of elevated blood serum concentrations of the Complement Factor H/Hs protein spot N4411 was accomplished with significantly higher sensitivity and specificity (FIG. **11**B, Table 11, Sensitivity=62.5%, Specificity=62.1%, cutoff value AD>270 ppm, ROC-P<0.0001). Conversely, when Apolipoprotein E4 protein spot N5302 was detected (N5302>0) in the blood serum, essentially no sensitivity and no specificity was obtained by measuring the concentration of Complement Factor H/Hs protein spot N4411 (FIG. **11**B, Table 11, Sensitivity=49.3% Specificity=49.3%, cutoff value AD>273 ppm, ROC-P<0.22). This is also an opposite effect to what was observed for Apolipoprotein E3 protein N3314 and Transthyretin "Dimer" protein spot N3307. Furthermore, similar results were obtained with Complement Factor Bb protein spot N7616 (FIG. **13**B, Table 13, N5302=0, Sensitivity 55.6%, Specificity=55.8%, cutoff value AD>237 ppm ROC-P<0.0040; vs. N5302>0, no Sensitivity 50.7%, no Specificity=49.3%, cutoff value AD>229 ppm ROC-P>0.06).

[0154] Thus, in active Alzheimer's disease, increased expression of Complement Factor H/Hs protein N4411 and Complement Factor Bb protein N7616 in blood serum has clinical diagnostic utility, when the detection or lack of detec-

tion in blood serum of the Alzheimer's disease risk gene allele Apo E ϵ4 protein product, Apolipoprotein E4 protein spot N5302 is also taken into account. Furthermore, the significantly up-regulated levels of Complement Factor H/Hs protein spot N4411 and Complement Factor Bb protein spot N7616 in Alzheimer's disease patients above age matched normal controls are found only in patients with no detectable Apolipoprotein E4 protein spot N5302 expression (N5302=0), is opposite to the effect of Apolipoprotein E4 protein spot N5302 expression on Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" Protein spot N3307. This indicates that reduced levels of Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" Protein spot N3307 reflect characteristics of one form of Alzheimer's disease (N5302>0), whereas increased levels of Complement Factor H/Hs protein spot N4411 and Complement Factor Bb protein spot N7616 are characteristics of the other form (N5302=0) of Alzheimer's disease, and this provides for complimentary diagnostic utilities.

[0155] In a preferred embodiment of the invention, combining the lack of detection or the detection, and the quantity of Apolipoprotein E4 protein spot N5302, with the concentrations of Apolipoprotein E3 protein spot N3314, Transthyretin "Dimer" protein spot N3307, Complement Factor H/Hs protein spot N4411, and Complement Factor Bb protein spot N7616 in blood serum wherein: Concentrations of Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein spot N3307 in blood serum that are significantly below the ranges of age matched normal controls when Apolipoprotein E4 protein spot N5302 is detected (N5302>0) and the concentrations of Apolipoprotein E4 protein spot N5302, are indicated for sensitive and specific detection of one form of Alzheimer's disease; and concentrations of Complement Factor H/Hs protein spot N4411 and Complement Factor Bb protein spot N7616 that are significantly above the range of age matched normal controls when Apolipoprotein E4 protein spot N5302 is not detected (N5302=0) are indicated for sensitive and specific detection of another form of Alzheimer's disease (N5302=0); and by detecting both of the types of Alzheimer's disease (wherein N5302=0 and N5302>0) with complementary characteristics, greater sensitivity and specificity is obtained for detection of Alzheimer's disease.

[0156] For the purposes of the preferred embodiment of this invention, the known activity of Complement Factor H/Hs protein spot N4411 in releasing Complement Factor Bb protein spot N7616 from the alternate Complement C3 Convertase, indicate significant differences in the mechanisms of neuronal degeneration between the two forms of Alzheimer's disease (N5302=0, N5302>0, FIG. **36**).

Complement C3c1, C3c2a, and C3dg

[0157] In the preferred embodiment of the invention, lack of detection, detection, detection and/or quantity of Apolipoprotein E4 protein spot N5302, as measured in blood serum (whether Apolipoprotein E4 protein spot N5302 concentration is >0 or=0), is determined and its effect upon the expression of Complement C3c1 phosphoprotein spot N7310, Complement C3c2a protein spot N9311, Complement C3dg protein spot N1511, and C3Sum (N7310+N9311+N1511), are also measured in blood serum. The two isoforms of Complement C3c protein (C3c1 and C3c2a) have the same amino acid sequence (ref. 17) derived from the same locus of Complement C3 parent precursor (SEQ ID NO. 6, FIG. **20**, amino acids 749-951). Phosphorylated Complement C3c1 protein spot N7310 (SEQ ID NO. 7) is derived from Complement C3 parent precursor tyrosine phosphorylated during its translation in response to auto-immune antibody stimulation of the neuronal Fc receptor, and non-phosphorylated Complement C3c2a protein spot N9311 (SEQ ID NO. 8) is derived from non-phosphorylated Complement C3 parent precursor in the Classical Complement Pathway of innate inflammation (refs. 17-37, FIGS. **20**, **34-37**).

[0158] Complement C3dg protein spot N1511 is also derived from the Complement C3 parent precursor, but downstream of the locus for Complement C3c1 and C3c2a (SEQ ID NO. 6, amino acids 955-1303). It arises when Complement iC3b is cleaved to make Complement C3c and Complement C3dg (SEQ ID NO. 10). Alternately, Complement C3dg protein spot N1511 (SEQ ID NO. 11) arises from alternate parent protein Similar to C3 (SEQ ID NO. 9, amino acids 902-1256).

[0159] As shown in FIG. **14**, Table 14, and FIG. **20**, Complement C3c1 phosphoprotein N7310 (FIG. **14**A, Table 14a), Complement C3c2a protein N9311 (FIG. **14**C, Table 14c), Complement C3dg protein N1511 (FIG. **14**B, Table 14b), and the C3Sum (N7310+N9311+N1511) (FIG. **14**D, Table 14d), were significantly up-regulated (ANOVA-P<0.0001, Table 14) in the blood serum of Alzheimer's and Parkinson's disease patients, compared to age matched normal controls. Complement C3c1 phosphoprotein spot N7310 is not up-regulated in blood serum of patients with AD-like and mixed dementias (FIG. **14**A, Table 14a), whereas Complement C3c2a protein spot N9311 is up-regulated in the blood serum of these patients (FIG. **14**C, Table 14c), and Complement C3dg protein spot N1511 is up-regulated to a lesser extent than Complement C3c2a protein spot N9311 in the blood serum of these patients (FIG. **14**B, Table 14b).

[0160] By ROC analysis, Complement C3c1 phosphoprotein spot N7310 (FIG. **15**A, Table 15a) Complement C3c2a protein spot N9311 (FIG. **15**C, Table 15c), Complement C3dg protein spot N1511 (FIG. **15**B, Table 15b), and the C3Sum (N7310+N9311+N1511) (FIG. **15**D, Table 15d), showed sensitivities and specificities of discrimination between 115 Alzheimer's disease patients and 75 age matched normal controls as follows:

[0161] 1. N7310: 55.9% sensitivity, 55.6% specificity (Table 15a, AD>273 ppm, ROC-P<0.0001);

[0162] 2. N1511: 60.9% sensitivity, 60.9% specificity (Table 15b, AD>105 ppm, ROC-P<0.0001);

[0163] 3. N9311: 53.9% sensitivity, 53.8% specificity (Table 15c, AD>272 ppm, ROC-P<0.0007);

[0164] 4. C3Sum: 55.1% sensitivity, 55.1% specificity (Table 15d, AD>710 ppm, ROC-P<0.0001).

[0165] Furthermore, in Alzheimer's disease patients, significantly up-regulated levels of Complement C3c1 phosphoprotein spot N7310, C3c2a protein spot N9311 and Complement C3dg protein spot N1511, and C3Sum, above age matched normal controls are found regardless of the detection (N5302>0), or lack of detection (N5302=0), of Apolipoprotein E4 protein spot N5302 expression (FIG. **16**; Table 16, ANOVA-P<0.0001). Also, the up-regulation was more pronounced with the Alzheimer's disease patients and age matched controls when Apolipoprotein E4 protein spot N5302 was not detected (N5302=0) in their blood serum than with the Alzheimer's disease patients and age matched controls when Apolipoprotein E4 protein spot N5302 was detected (N5302>0) in their blood serum.

[0166] 1. N7310: N5302=0, AD=323% of AMC; N5302>0, AD=269% of AMC (Table 16a)

[0167] 2. N1511: N5302=0, AD=511% of AMC; N5302>0, AD=338% of AMC (Table 16b)

[0168] 3. C3Sum: N5302=0, AD=295% of AMC; N5302>0, AD=256% of AMC (Table 16b)

[0169] The one exception was Complement C3c2a protein spot N9311, where the up-regulation was essentially to the same extent, regardless of whether Apolipoprotein E4 protein spot N5302 was detected in their blood serum.

[0170] N9311: N5302=0, AD=196% of AMC; N5302>0, AD=206% of AMC (Table 16a)

[0171] Using ROC analysis (FIG. **17**, Table 17), these protein biomarkers demonstrate discrimination of AD from age matched normal controls with:

[0172] 1. N7310: N5302=0; 59.0% sensitivity, 59.0% specificity (Table 17a, AD>309 ppm, ROC-P<0.0006);

[0173] 2. N7310: N5302>0; 59.2% sensitivity, 59.4% specificity (Table 17a, AD>273 ppm, ROC-P<0.0002);

[0174] 3. N1511: N5302=0, 63.2% sensitivity, 62.8% specificity (Table 17b, AD>107 ppm, ROC-P<0.0001);

[0175] 4. N1511: N5302>0, 58.2% sensitivity, 58.0% specificity (Table 17b, AD>106 ppm, ROC-P<0.0001).

[0176] 5. C3Sum: N5302=0, 56.3% sensitivity, 56.4% specificity (Table 17b, AD>743 ppm, ROC-P<0.0001);

[0177] 6. C3Sum: N5302>0, 58.2% sensitivity, 58.0% specificity (Table 17b, AD>635 ppm, ROC-P<0.0001).

[0178] Again, the only exception in Complement C3c2a protein spot N9311, which only showed sensitivity and specificity, when N5302>0:

[0179] 1. N9311: N5302=0, 52.1% no sensitivity, 51.9% no specificity (Table 17b, AD>107 ppm, ROC-P<0.03);

[0180] 2. N9311: N5302>0, 58.2% sensitivity, 58.0% specificity (Table 17b, AD>106 ppm, ROC-P<0.0006).

[0181] When the severity of Alzheimer's disease is taken into account (MMSE scores) (FIGS. **18**, **19**), differences in blood serum concentration vs. Alzheimer's disease severity were found between Complement C3c1 protein spot N7310 and Complement C3c2a protein spot N9311, and between the detection, or the lack of detection, of Apolipoprotein E4 protein spot N5302.

[0182] In patients with no detectable levels of Apolipoprotein E4 protein spot N5302, blood serum concentration of Complement C3c1 protein spot N7310 is 14 fold higher than age matched normal controls (FIG. **18**A, N5302=0, ANOVA-P<0.0001, vs. FIG. **18**C). Furthermore, that level declines in a statistically significant fashion with increasing of AD severity (FIG. **18**A, Decreasing MMSE=Increasing severity of dementia, Linear Regr.-P<0.0001).

[0183] Similarly, in patients with no detectable levels of Apolipoprotein E4 protein spot N5302, (FIG. **19**A; N5302=0) expression of Complement C3dg protein spot N1511 in blood serum is 12 fold higher than age matched normal controls (FIG. **19**A, N5302=0, ANOVA-P<0.0001, vs. FIG. **19**B) and the level declines in a statistically significant fashion with increasing of AD severity (FIG. **19***a*, Decreasing MMSE=Increasing severity of dementia, Linear Regr.-P<0.002).

[0184] On the other hand, in patients with no detectable Apolipoprotein E4 protein spot N5302, the expression of Complement C3c2a protein spot N9311 is higher (5 fold) than age matched normal controls (FIG. **18**D, N5302=0, ANOVA-P<0.0001 vs. 18f), but there is no statistically significant correlation in expression levels of N9311 with increasing of AD severity (FIG. **18**D, N5302=0, Linear Regr.-P>0.080).

[0185] In patients with detectable Apolipoprotein E4 protein spot N5302, expression of Complement C3c1 protein spot N7310 is also higher (5 fold) than age matched normal controls (FIG. **18**B, N5302>0, ANOVA-P<0.0001, vs. FIG. **18**C), but there is no statistically significant correlation in expression levels of N7310 with increasing AD severity (FIG. **19**A, Decreasing MMSE=Increasing severity of dementia, Linear Regr.-P>0.80).

[0186] However, expression of Complement C3c2a protein spot N9311 is not significantly higher than age matched controls in mild AD (FIG. **18**E, N5302>0, vs. FIG. **18**F) but in moderate and severe AD, the levels are 5 fold higher than age matched normal control and is in a significant correlation with increasing of AD severity in patients with detectable Apolipoprotein E4 protein spot N5302, (FIG. **18**E, N5302>0, ANOVA-P<0.0001, Linear Regr.-P<0.040, vs. FIG. **18**F).

[0187] Similar to Complement C3c2a protein spot N9311, in patients with detectable Apolipoprotein E4 protein spot N5302, expression of Complement C3dg protein spot N1511 is not significantly higher than age matched controls in mild AD (FIG. **19**C, N5302>0, vs. FIG. **19**B) but the levels are 12 fold higher in moderate and severe AD in a statistically significant correlation with increasing AD severity (FIG. **19**C, N5302>0, ANOVA-P<0.0001, Linear Regr.-P<0.030, vs. FIG. **19**B).

[0188] In a preferred embodiment of the invention, Complement C3c1 protein N7310 blood serum concentration significantly above age matched normal controls is an indication for:

[0189] 1. Early detection of AD and monitoring of AD severity, in patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0), and for

[0190] 2. Early detection of AD but not for monitoring of AD severity, in patients with detectable Apolipoprotein E4 protein spot N5302 (N5302>0).

[0191] Also in the preferred embodiment of the invention, concentrations of Complement C3c2a protein spot N9311 significantly above the level of age matched normal controls is an indication for:

[0192] 3. Early detection of AD but not for monitoring of AD severity, in patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0), and for

[0193] 4. Monitoring of AD severity but not for early detection of AD, in patients with detectable Apolipoprotein E4 protein spot N5302 (N5302>0).

[0194] Also in the preferred embodiment of the invention, the effect of detection, or a lack of detection of Apolipoprotein E4 protein spot N5302 expression, as measured in blood serum (whether Apolipoprotein E4 protein spot N5302 concentration is >0 or =0), in association with the expression of Complement C3dg protein spot N1511 is determined. Complement C3dg protein spot N1511 (Table 12, SEQ ID NO. 10) consists of a different amino acid sequence derived from a sequence domain downstream of the locus shared by Complement C3c1 protein spot N7310 and C3c2a protein spot N9311, of Complement C3 (Table 8, SEQ ID NO. 6) parent precursor and also derived from as an alternative isoform (Table 13, SEQ ID NO. 11) derived from an alternate parent precursor Similar to C3 (Table 11, SEQ ID NO. 9).

[0195] Thus, in a preferred embodiment of the invention, the significantly higher level of blood serum concentration of Complement C3dg protein spot N1511 in Alzheimer's disease patients than that of aged matched normal controls is an indication for:

[0196] 1. Early detection of AD and monitoring of AD severity, in patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0; decreasing blood serum concentration of Complement C3dg protein spot N1511 with increasing Alzheimer's disease severity), and for

[0197] 2. Monitoring of AD severity but not for early detection of AD, in patients with detectable Apolipoprotein E4 protein spot N5302 (N5302>0; increasing blood serum concentration of Complement C3dg protein spot N1511 with increasing Alzheimer's disease severity).

The Haptoglobin HP-1 Proteins N1514, N2401, N2407, and N3409

[0198] Haptoglobin HP-1 Protein spots N1514, N2401, N2407, and N3409 contain the same amino acid sequence (SEQ ID NO. 19), but differ in their electrophoretic mobility in 2D gel electrophoresis (FIG. **2**). They are up-regulated in parallel in the blood serum of patients with Alzheimer's disease and patients with AD-like and mixed dementias, but not in patients with Parkinson's disease, as compared to age matched normal controls (FIG. **21**).

[0199] As shown in FIG. **21**D, Differential expression of Haptoglobin HP-1 protein spot N3409 between Alzheimer's disease patients and Parkinson's disease patients is particularly pronounced. In Alzheimer's disease patients, Haptoglobin HP-1 protein spot N3409 is up-regulated from age matched normal controls, whereas in Parkinson's disease patients, Haptoglobin HP-1 protein spot N3409 is down regulated from age matched normal controls. This provides for significantly higher sensitivity and specificity for distinguishing between these two diseases based on the concentration of Haptoglobin HP-1 protein spot N3409 (71.9% and 72.8%, respectively, FIG. **21**E)

[0200] In a preferred embodiment of the invention, the concentrations of Haptoglobin HP-1 protein spots N1514, N2401, N2407, and N3409 and their sum (HP-1 Total Proteins, FIG. **22**) are employed. HP-1 Total Proteins are up-regulated in a statistically significant manner in the blood serum of patients with Alzheimer's disease, and patients with AD-like and Mixed dementias, but not in patients with Parkinson's disease, as compared to age matched normal controls (FIG. **22**A, ANOVA-P<0.0030).

[0201] Using the ROC analysis, the Total of HP-1 Protein spots showed sensitivities and specificities of discrimination between 115 Alzheimer's disease patients and 75 age matched normal control individuals as follows:

HP-1 Total Proteins: 56.2% sensitivity, 56.0% specificity (Table 18, AD>30136 ppm, ROC-P<0.0001).

[0202] Furthermore, in Alzheimer's disease patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0), blood serum concentrations of Haptoglobin HP-1 protein spots N1514, N2401, N2407, N3409, and HP-1 Total Proteins are significantly higher than age matched normal controls (FIG. **23**, FIG. **25**A, N5302=0, ANOVA-P<0.0001).

[0203] On the other hand, in Alzheimer's disease patients and age matched normal controls with detectable blood serum levels of Apolipoprotein E4 protein spot N5302 (N5302>0), Haptoglobin HP-1 Proteins spots N1514, N2401, N2407, N3409, and HP-1 Total Proteins are not significantly different from the levels of age matched controls (FIG. **23**, FIG. **25**A, N5302=0, ANOVA-P>0.7). However, the concentrations of Haptoglobin HP-1 Proteins N1514, N2401, N2407, N3409, and HP-1 Total Proteins in Alzheimer's disease patients and age matched normal controls with detectable levels of Apolipoprotein E4 protein spot N5302 (N5302>0 are both significantly higher than age matched normal controls with no detectable levels of Apolipoprotein E4 protein N5302 (N5302=0) (FIGS. **23**-**25**).

[0204] ROC analysis demonstrated specificity and sensitivity for separation between Alzheimer's disease patients and age matched normal controls with no detectable Apolipoprotein E4 protein spot N5302, and no specificity nor sensitivity for separation between Alzheimer's disease patients and age matched normal controls with detectable Apolipoprotein E4 protein spot N5302 (FIG. **25***b*) as follows:

[0205] 1. HP-1 Total Proteins: N5302=0, 64.6% sensitivity, 64.7% specificity (Table 19, AD>30216 ppm, ROC-P<0.0001);

[0206] 2. HP-1 Total Proteins: N5302>0, 44.8% sensitivity, 44.9% specificity (Table 19, AD>30216 ppm, ROC-P<0.0001);

[0207] Thus, in a preferred embodiment of the invention, the significantly higher level of blood serum concentration of Haptoglobin HP-1 Protein spots N1514, N2401, N2407, N3409, and HP-1 Total Proteins (N1514+N2401+N2407+N3409) in Alzheimer's disease patients than that of aged matched normal controls is an indication for:

[0208] 3. Detection of AD in patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0), and

[0209] 4. Discrimination of patients with AD from patients with PD.

[0210] 5. But not for detection of AD in patients with detectable levels of Apolipoprotein E4 protein spot N5302 (N5302>0), Inter-alpha-trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307

[0211] As shown in FIG. **26** and Table 20, Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307 is significantly up-regulated in the blood serum of patients with Alzheimer's disease (ANOVA-P<0.0001), Parkinson's disease, and with Stroke related, and Mixed dementias, and conversely was significantly down regulated in blood serum of patients with non-stroke related dementias, including: Frontotemporal dementia, Lewy body dementia, Corticalbasal Ganglionic degeneration, alcohol related dementia, and semantic dementia, as compared to age matched normal controls (FIG. **26**A, Table 20).

[0212] Using ROC analysis (FIG. **26**B, Table 20), the blood serum concentration of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307 distinguishes between 115 patients with Alzheimer's disease and 75 age matched normal controls (FIG. **26**B) as follows: N2307: 58.0% sensitivity, 58.2% specificity (Table 20, AD>210 ppm, ROC-P<0.0001).

[0213] In Alzheimer's disease patients and age matched controls with and without detectable blood serum levels of Apolipoprotein E4 protein spot N5302, the expression levels of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307 in blood serum is significantly higher than complementary age matched normal controls (FIG. **27**A, N5302=0, Table 21a, ANOVA-P<0.0001; ANOVA-P>0.06).

[0214] Using an ROC analysis (FIG. **27**B, Table 21b), blood serum concentration of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307 demonstrated sensitivity and specificity for diagnosis of AD from age matched normal controls when Apolipoprotein E4 pro-

tein spot N5302 was not detected (N5302=0) but not when Apolipoprotein E4 protein spot N5302 was detected (N5302>0) in blood serum:

[0215] 1. N2307: N5302=0; 61.8% sensitivity, 61.5% specificity (Table 21b, AD>211 ppm, ROC-P<0.0001);

[0216] 2. N2307: N5302>0, 50.7% sensitivity, 50.7% specificity (Table 21b, AD>224 ppm, ROC-P>0.14).

[0217] Furthermore, as shown in FIG. **28**, expression of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307 in blood serum is significantly higher (2.7 fold) than the age matched normal controls in mild Alzheimer's disease patients without detectable blood serum level of Apolipoprotein E4 protein spot N5302 (FIG. **28**A, N5302=0; ANOVA-P<0.0001 vs. FIG. **28**B), in this group of patients, there is statistically significant decline in expression levels of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307, in correlation with increasing the severity of AD. These results are similar to that of Complement C3c1 protein spot N7310 (FIG. **28**A Linear Regr-P vs. FIG. **28**B, compare with FIG. **18**A, N7310, N5302=0).

[0218] Also, as shown in FIG. **28**, expression of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307 in blood serum is also significantly higher (2.2 fold) than the age matched normal controls in mild Alzheimer's disease in patients with detectable Apolipoprotein E4 protein spot N5302. In this group of patients, there is no significant correlation with the increased severity of AD (FIG. **28**C, N5302>0 vs. ANOVA-P<0.0001, Linear Regr.-P>0.20 vs. FIG. **28**B). These results are also similar to that of Complement C3c1 protein N7310 (compare with FIG. **18**B, N5302>0).

[0219] Thus in a preferred embodiment of the invention, as in the case of Complement C3c1 protein N7310, the significantly higher level of the blood serum concentrations of Inter alpha trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307, in Alzheimer's disease patients than that of the age matched normal controls, is an indication for:

[0220] 1. Early detection of AD and monitoring of AD severity, in patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0), and for

[0221] 2. Early detection of AD but not for monitoring of AD severity, in patients with detectable Apolipoprotein E4 spot protein N5302 (N5302>0); Immunoglobulin Light Chain Protein N6224 and Apolipoprotein A-IV Protein spot N2502

[0222] As shown in FIGS. **29** and **31**, Tables 22 and 24, Immunoglobulin Light Chain protein spot N6224 and Apolipoprotein A-IV protein spot N2502 are both significantly down-regulated in the blood serum of patients with Alzheimer's disease, Parkinson's disease, and AD-Like and Mixed dementias, as compared to age matched normal controls (FIG. **29**A, Table 22, N6224, ANOVA-P<0.0002; FIG. **31**A, Table 24, N2502, ANOVA-P<0.0001).

[0223] Using the ROC analysis (FIG. **29**B, Table 22, FIG. **31**B, Table 24), the blood serum concentrations of both Immunoglobulin Light Chain protein spot N6224 and Apolipoprotein A-IV protein spot N2502 distinguish between 115 patients with Alzheimer's disease and 75 age matched normal controls (FIG. **29**B, N6224, FIG. **31**B, N**2502**) as follows:

[0224] 1. N6224: 59.7% sensitivity, 59.6% specificity (Table 22b, AD<368 ppm, ROC-P<0.0001).

[0225] 2. N2502: 59.1% sensitivity, 59.1% specificity (Table 23b, AD<2465 ppm, ROC-P<0.0001).

[0226] Down-regulated blood serum levels of Immunoglobulin Light Chain protein spot N6224 and Apolipoprotein A-IV Protein spot N2502 in Alzheimer's disease patients below that of age matched normal controls are found regardless of whether Apolipoprotein E4 protein spot N5302 was detected or not in blood serum; although more significant in the case of N5302>0 for Immunoglobulin Light Chain protein spot N6224 (FIG. **30**A, N6224, Table 23, N5302=0, ANOVA-P>0.08; N5302>0, ANOVA-P<0.0007), and more significant in the case of N5302=0 for Apolipoprotein A-IV protein spot N2502 (FIG. **32**A, N2502, Table 25, N5302=0, ANOVA-P<0.0003; N5302>0, ANOVA-P<0.03).

[0227] Using the ROC analysis (FIG. **30**B, Table 23, FIG. **32**B, Table 25), blood serum levels of Immunoglobulin Light Chain protein spot N6224 and Apolipoprotein A-IV protein spot N2502 distinguish between Alzheimer's disease patients and age matched normal controls whether Apolipoprotein E4 protein spot N5302 was detected or not in blood serum as follows:

[0228] N6224: N5302=0; 56.9% sensitivity, 57.1% specificity (Table 23, AD<368 ppm, ROC-P<0.0003);

[0229] N6224: N5302>0, 62.7% sensitivity, 62.3% specificity (Table 23, AD<378 ppm, ROC-P<0.0001).

[0230] N2502: N5302=0; 58.3% sensitivity, 58.3% specificity (Table 25, AD<2412 ppm, ROC-P<0.0001);

[0231] N2502: N5302>0; 62.2% sensitivity, 62.3% specificity (Table 25, AD<2588 ppm, ROC-P<0.0003);

[0232] Thus in a preferred embodiment of the invention, the significantly low blood serum levels of Immunoglobulin Light Chain protein spot N6224 and Apolipoprotein A-IV protein spot N2502 in Alzheimer's disease patients than that of the age matched normal controls is an indication for: Detection of AD in patients with no detectable Apolipoprotein E4 protein spot N5302 (N5302=0), and for Detection of AD in patients with detectable Apolipoprotein E4 protein spot N5302 (N5302>0);

[0233] As illustrated in Table 26, in a preferred embodiment of the invention, when the blood serum concentrations of Apolipoprotein E4 protein spot N5302 and Apolipoprotein E3 protein spot N3314, Complement Factor H/Hs protein spot N4411, Complement Factor Bb protein spot N7616, Complement C3c1 phosphoprotein spot N7310, Complement C3c2a protein spot N9311, Complement C3dg protein spot N1511, Haptoglobin HP-1 Total Proteins (N1514+N2401+N2407+N3409), Inter alpha trypsin inhibitor heavy chain (H4) related 35 KD protein spot N2307, Immunoglobulin Light Chain Protein spot N6224 and Apolipoprotein A-IV protein spot N2502 are all combined into a multivariate linear discriminant function to distinguish between all 115 Alzheimer's disease patients and all 75 age matched normal controls, a sensitivity of 69.6% and a specificity of 84.4% are obtained. However, when the Alzheimer's disease patients and age-matched normal control subjects are separated into two groups, based on whether Apolipoprotein E4 protein spot N5302 is detected or not in the blood serum, a sensitivity of 82.3% and a specificity of 82.7% are obtained when the results are combined after the discriminant analysis (Table 26). These results underscore the importance of differentiation between two types of Alzheimer's disease patients for the purpose of better sensitivity during diagnosis of the disease.

[0234] In a preferred embodiment of the invention separate linear discriminant functions are performed for those in whom Apolipoprotein E4 protein spot N5302 is detected in blood serum (N5302>0) and those in whom Apolipoprotein

E4 protein spot N5302 is not detected in blood serum (N5302=0). In each linear discriminant function, Alzheimer's disease patients and Age matched normal controls are distinguished from one another. Also in the preferred embodiment of the invention, the linear discriminant function is generated with the addition of concentrations of other blood serum protein biomarkers, for example, one or more of Apolipoprotein E3 protein spot N3314, Complement Factor H/Hs protein spot N4411, Complement Factor Bb protein spot N7616, Complement C3c1 phosphoprotein spot N7310, Complement C3c2a protein spot N9311, Complement C3dg protein spot N1511, Haptoglobin HP-1 individual and Total of protein spots (N1514+N2401+N2407+N3409), Inter alpha trypsin inhibitor heavy chain (H4) related 35 KD protein spot N2307, Immunoglobulin Light Chain protein spot N6224 and Apolipoprotein A-IV protein spot N2502.

[0235] When separate discriminant functions are performed in the manner of the invention (Table 26) and the results are then combined by adding the true positives together, the true negatives together, the false positives together, and the false negatives together, that were generated by the separate discriminant functions, this results in clinically significant sensitivity and specificity (Table 26, Sensitivity 82.3%, Specificity 82.7%).

[0236] Each step of sensitivity and specificity improvements for diagnosis of Alzheimer's disease attained by the invention are shown in FIG. **33**. Furthermore, the invention is built by leveraging individual biomarkers with individual utilities that fall into types as illustrated in FIG. **34** (Types 1-5) based on their relationship to the Alzheimer's disease and the ways in which the disease manifests.

[0237] FIGS. **35-36** illustrate the disease pathways indicated by the abnormal changes in concentration of some of these blood serum protein biomarkers: Apolipoprotein E3 (Apo E3); Transthyretin Dimer (TTD); Inter-alpha-trypsin inhibitor heavy chain (H4) related 35 KD protein (ITI(H4)RP 35 KD); Complement C3c1 tyrosine phosphoprotein (C3c1 (p)); Complement C3c2a protein (C3c2a); Complement C3dg protein (C3dg); Complement Factor H protein (Factor H), and Complement Factor Bb protein (Factor Bb); all of which have been disclosed before in connection with inventions for diagnosis and monitoring of neurodegenerative diseases (refs. 17-19; U.S. Utility patent application Ser. No. 11/507,337 filed Aug. 22, 2006 and entitled "Assay for Diagnosis and Therapeutics Employing Similarities and Differences in Blood Serum Concentrations of 3 forms of Complement C3c and Related Protein Biomarkers between Amyotrophic Lateral Sclerosis and Parkinson's Disease" by inventors Ira L. Goldknopf et al., U.S. Provisional Patent Application Ser. No. 60/901,467 filed Feb. 16, 2007 and entitled "Forty Seven (47) Protein Biomarkers for Neurodegenerative Diseases," by inventors Ira L. Goldknopf et al., U.S. Utility patent application Ser. No. 12/069,807 filed Feb. 14, 2008 and entitled "Forty Seven (47) Protein Biomarkers for Neurodegenerative Diseases," by inventors Ira L. Goldknopf, U.S. Utility patent application Ser. No. 11/602,814 filed 11/21/06 and entitled "An Inter-Alpha Trypsin Inhibitor Heavy Chain (H4) Related Protein as a Biomarker of Alzheimer's Disease," by inventors Ira L. Goldknopf, et al, U.S. Utility patent application Ser. No. pending filed Aug. 29, 2007 and entitled "A Complement Factor H Protein as a Biomarker of Parkinson's Disease," by inventors Ira L. Goldknopf, et al., U.S. Utility patent application Ser. No. pending filed Sep. 5, 2007 and entitled "An Apolipoprotein E3 Protein as a Biomarker of Parkinson's Disease," by inventors Ira L. Goldknopf, et al., and herein all incorporated by reference).

[0238] In this preferred embodiment of the invention we have compared these changes as a function of the detection (FIG. **35**A, B; FIG. **36**B) or lack of detection (FIG. **35**C, D; FIG. **36**A) of Apolipoprotein E4 protein in the blood serum of the patients. We have found parallel, specific differences between Alzheimer's disease patients and age matched normal controls in the blood serum concentrations of two biomarkers that are closely related to the Apo E ϵ4 gene allele protein product, Apolipoprotein E4 protein spot N5302: Apo E ϵ3 gene allele protein product, Apolipoprotein E3 protein spot N3314; and Transthyretin "Dimer" protein spot N3307.

[0239] When both the AD patients and controls had detectable blood serum levels of Apolipoprotein E4 protein spot N5302, the protein biomarkers Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein spot N3307 were markedly reduced in blood serum concentration in the Alzheimer's disease patients (FIG. **34** Type 1, FIG. **35**A, FIG. **36**B; N5302>0). However, the reductions in blood serum concentrations of these two protein biomarkers were much less pronounced when both AD patients and controls had no detectable blood serum levels of Apolipoprotein E4 protein (FIG. **34** Type 1, FIG. **35**C, FIG. **36**A, N5302=0).

[0240] Those patients for which the Apolipoprotein E4 protein spot N5302 was detected in their blood serum (Apo E4>0, FIG. **35**A, B, FIG. **36**B) must have at least 1 copy of the Apo E ϵ4 gene allele in their genome, and the allele is expressed as a protein (spot N5302). To uncover the significance of these differences, we incorporate the well established findings that persons who carry the gene allele Apo E ϵ4 have substantially higher risk of developing Alzheimer's disease and other dementias (6, 38-39), and also have higher levels of the Amyloid plaque forming Aβ-42 and 1-40 peptides, higher levels of the Amyloid plaques, of the neurofibrillary tangle forming hyper-phosphorylated Tau, and of the neurofibrillary tangles than individuals without the Apo E ϵ4 allele. All of these data correlate with the development of AD. Furthermore, these differences are reflected in normal controls and even greater in Alzheimer's disease patients (40-42).

Neuronal Degeneration in Alzheimer's Disease Patients with Detectable Apolipoprotein E4 Protein in Blood Serum

[0241] The marked reduction in the blood serum concentration of soluble Apolipoprotein E3 protein spot N3314 and Transthyretin "Dimer" protein spot N3307, in patients with detectable blood serum Apolipoprotein E4 protein spot N5302 (FIG. **35**A, FIG. **36**B, N5302>0) is attributable to their known incorporation into insoluble Amyloid plaques and neurofibrillary tangles (17, 18, 43), which are also known to be increased in Apo E ϵ4 gene allele positive AD patients (40-42). The reduced level (46%, compared to AMC) of soluble Apolipoprotein E3 protein in this type of patients would also attenuate the known neuro-protective mechanisms against oxidative stress that are also known to be facilitated by soluble Apolipoprotein E3. These include: 1) maintenance of intra-neuronal cholesterol; and metabolism of peroxidized lipids; both mediated by the Apo E receptor; and NMDA receptor mediated glutamate/calcium homeostasis, (6, 17-19, 40-46; FIGS. **35**A, **36**B, **37**B).

[0242] Such diminished neuroprotection, is coincident with known markedly increased oxidative stress in Apo E ϵ4 allele positive AD (47-52), resulting in uncontrolled neuronal oxidative stress and apoptosis as the primary neurodegenera-

tive pathway driving AD in Apolipoprotein E4 protein spot N5302 positive patients (FIGS. **35**A; **36**B).

[0243] In Apolipoprotein E4 protein spot N5302 positive patients (N5302>0), we have also found elevated blood serum levels of other protein biomarkers (FIGS. **34**, **35**B; **36**B) indicative of a secondary neurodegenerative pathway of inflammation. Two of these proteins were previously found associated with localized acquired auto-immune inflammation in sporadic ALS and Parkinson's disease (17-19, and U.S. Utility patent application Ser. No. 11/507,337 filed Aug. 22, 2006 and entitled "Assay for Diagnosis and Therapeutics Employing Similarities and Differences in Blood Serum Concentrations of 3 forms of Complement C3c and Related Protein Biomarkers between Amyotrophic Lateral Sclerosis and Parkinson's Disease" by inventors Ira L. Goldknopf et al. and herein incorporated by reference). This included elevated blood serum levels of Complement C3c1 tyrosine phosphoprotein spot N7310 and Complement Factor Bb protein spot N7616. In addition, in Apolipoprotein E4 protein spot N5302 positive Alzheimer's disease patients, there were delayed elevations of blood serum concentrations, i.e. in severe AD, of Complement C3c2a protein (C3c2a) spot N9311; Complement C3dg protein (C3dg) spot N1511; and Inter-alpha-trypsin inhibitor heavy chain (H4) related 35 KD protein (ITI (H4) RP 35 KD) spot N2307, known systemic innate inflammatory response associated proteins (Ref. 17-19). In AD, this secondary innate inflammatory pathway is most likely due to the known enhanced induction and secretion of inflammatory cytokines, particularly IL-6 in response to increased Amyloidosis and neurofibrillary tangles in Apo E ϵ4 positive AD (FIGS. **35**B, **36**B; refs. 21-25, 31-37, 53, 54).

[0244] Thus in the preferred embodiment of the invention, in Alzheimer's disease patients with detectable Apolipoprotein E4 protein spot N5302 in their blood serum (FIG. **35** A, B; N5302>0), the predominant or primary mechanism driving neurodegeneration is Aβ/NFT-induced oxidative stress leading to neuronal apoptosis, with a secondary immune inflammatory response due to delayed Aβ/NFT-induced pro-inflammatory cytokine induction.

Neuronal Degeneration in Alzheimer's Disease Patients with No Detectable Apolipoprotein E4 Protein in Blood Serum

[0245] A different pattern emerged when AD patients with no detectable Apolipoprotein E4 protein spot N5302 in their blood serum were compared to a group of normal controls, also with no detectable Apolipoprotein E4 protein spot N5302 in their blood serum (FIG. **35** C, D; N5302=0). In these AD patients, there was little reduction (20%, compared to AMC) in blood serum concentration of Apolipoprotein E3 protein spot N3314, leaving intact the neuroprotective maintenance of cholesterol homeostasis and attenuation of oxidative stress, known to be associated with increased concentration of Apolipoprotein E (6, 17-19, 40-46, 55-59). It is also well known that accumulation of Aβ and NFTs, and the concomitant generation of oxidative stress intermediates is much less in AD patients lacking the Apo E ϵ4 allele than in those that have the allele (40-42, 47-51). This combination of factors should markedly attenuate the oxidative stress related apoptosis and the Aβ/NFT-induced pro-inflammatory cytokine induction of inflammation demonstrated in Apolipoprotein E4 protein spot N5302 positive Alzheimer's patients (FIG. **35**B).

[0246] Nevertheless, Apolipoprotein E4 protein spot N5302 positive Alzheimer's disease patients are also undergoing neurodegeneration. The answer lies in the additional biomarkers of acquired immune and innate inflammation (FIG. **1**D). These include a pattern of pronounced elevation in the blood serum concentration of: Complement C3c1 phosphoprotein spot N7310 (8, 9, 38-40); paralleled by similar elevations in Complement C3dg protein spot N1511 and Inter-a-trypsin inhibitor heavy chain H4 related 35 KD protein spot N2307; all three of which are at maximally high levels in mild AD, somewhat less high in moderate AD, and slightly high in severe AD patients' blood serum (FIG. **35**D; N5302=0).

[0247] Also the blood serum levels of innate immune inflammatory biomarkers (8, 9, 38-40) Complement C3c2a protein spot N9311, Complement Factor H/Hs protein spot N4411 and Complement Factor Bb protein spot N7616 were all elevated to moderately high levels in mild, moderate and severe AD.

[0248] Thus, in the preferred embodiment of the invention, in the Alzheimer's disease patients with no detectable Apolipoprotein E4 protein spot N5302 in their blood serum (N5302=0), the apoptosis pathway is inhibited and auto-immune inflammation is the predominant pathway driving neuronal degeneration in these patients.

Analogies with Other Neurodegenerative Diseases

[0249] As illustrated in Table 27, our findings with blood serum biomarkers in Alzheimer's disease were analogous to our previous findings with the same blood serum protein biomarkers in ALS (17, 18): familial ALS resembles Apolipoprotein E4 protein spot N5302 positive Alzheimer's disease; and sporadic ALS resembles Apolipoprotein E4 protein spot N5302 negative Alzheimer's disease. Thus the expression of an Apo E ϵ4 allele protein (N5302>0; a single amino acid mutation in 14% of the population), which signifies higher risk of Alzheimer's disease (5, 6, 38, 39), as well as cognitive deficits in "normal" aged individuals (38), leads to a primary oxidative stress driven apoptotic Alzheimer's disease phenotype, just as does the expression of the ALS risk genetic mutant Superoxide dismutase protein in familial ALS (17, 18) Similarly, in Alzheimer's disease patients not expressing the Apo E ϵ4 allele protein (N5302=0), an immune inflammatory mechanism is responsible for driving neurodegeneration, just as is the case in the absence of the Superoxide dismutase mutations in sporatic ALS (17, 18) and in Parkinson's disease (17, 18, 63).

Applications for Similarities and Differences in Neurodegenerative Diseases

[0250] Proteins in the blood serum can tell us what disease pathways and mechanisms of neuronal degeneration are active in the patients. We have illustrated this with mechanistic differences, as indicated by blood serum proteomics, between two different types of Alzheimer's disease, and previously between two different types of ALS (17, 18). The mechanisms of neurodegeneration that display variations between two forms of each disease are oxidative stress, apoptosis, and immune inflammatory phagocytosis. These familial vs. sporadic disease variations in mechanisms are demonstrated both by Alzheimer's disease and ALS (Table 28). However, when additional blood serum proteins are brought into the analysis, disease specific differences emerge, with capabilities for differential diagnosis between diseases with similar symptoms (Table 29, ref. 19), implying additional

disease specific mechanistic differences, which will ultimately lead to differential treatment and personalized medicine (Table 28, ref. 19).

Additional Embodiments

[0251] The blood serum samples may also be subjected to various other techniques known in the art for separating and quantitating proteins. Such techniques include, but are not limited to: gel filtration chromatography, ion exchange chromatography, reverse phase chromatography, affinity chromatography (typically in an HPLC or FPLC apparatus), affinity capture, one dimensional gel or capillary electrophoresis, or any of the various centrifugation techniques well known in the art. Certain embodiments would also include a combination of one or more chromatography; electrophoresis or centrifugation steps combined via electrospray or nanospray with mass spectrometry or tandem mass spectrometry of the proteins themselves, or of a total digest of the protein mixtures. Certain embodiments may also include surface enhanced laser desorption mass spectrometry or tandem mass spectrometry, or any protein separation technique that determines the pattern of proteins in the mixture, either as a one-dimensional, two-dimensional, three-dimensional or multi-dimensional protein pattern, and/or the pattern of protein post synthetic modifications or different isoforms of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor protein, are used.

[0252] Quantitation of a protein by antibodies directed against that protein is well known in the field. The techniques and methodologies for the production of one or more antibodies to an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, are routine in the field and are not described in detail herein.

[0253] As used herein, the term antibody is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE. Generally, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting.

[0254] Monoclonal antibodies (MAbs) are recognized to have certain advantages, e.g., reproducibility and large-scale production, and their use is generally preferred. The invention thus provides monoclonal antibodies of human, murine, monkey, rat, hamster, rabbit, chicken, or other animal origin. Due to the ease of preparation and ready availability of reagents, murine monoclonal antibodies are generally preferred. However, human auto antibodies or "humanized" antibodies are also contemplated, as are chimeric antibodies from mouse, rat, or other species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and engineered antibodies and fragments thereof.

[0255] The term "antibody" thus also refers to any antibody-like molecule that has a 20 amino acid antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')2, single domain antibodies (DABS), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means of preparing and characterizing antibodies are also well known in the art (See, e.g., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference).

[0256] Antibodies to an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein may be used in a variety of assays in order to quantitate the protein in serum samples, or other fluid or tissue samples. Well known methods include immunoprecipitation, antibody sandwich assays, ELISA and affinity chromatography methods that include antibodies bound to a solid support. Such methods also include micro arrays of antibodies or proteins contained on a glass slide or a silicon chip, for example.

[0257] It is contemplated that arrays of antibodies to an Apolipoprotein E3 protein, or peptides derived from an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, may be produced in an array and contacted with the serum samples or protein fractions of serum samples in order to quantitate the blood serum concentrations of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein. The use of such micro arrays is well known in the art and is described, for example in U.S. Pat. No. 5,143,854 incorporated herein by reference.

[0258] The present invention includes a screening assay for neurodegenerative disease based on the up-regulation and/or down-regulation of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein expression. One embodiment of the assay will be constructed with antibodies to an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein. One or more antibodies targeted to antigenic determinants of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, will be spotted onto a surface, such as a polyvinyl membrane or glass slide. As the antibodies used will each recognize an antigenic determinant of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor

Heavy Chain (H4) related protein, incubation of the spots with patient samples will permit attachment of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, to the antibody.

[0259] The binding of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, can be reported using any of the known reporter techniques including radioimmunoassay (RIA), stains, enzyme linked immunosorbant assays (ELISA), and sandwich ELISAs with a horseradish peroxidase (HRP)-conjugated second antibody also recognizing an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, the pre-binding of fluorescent dyes to the proteins in the sample, or biotinylating the proteins in the sample and using an HRP-bound streptavidin reporter. The HRP can be developed with a chemiluminescent, fluorescent, or colorimetric reporter. Other enzymes, such as luciferase or glucose oxidase, or any enzyme that can be used to develop light or color can be utilized at this step.

[0260] All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods, and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention.

[0261] More specifically, it is well recognized in the art that the statistical data, including but not limited to the mean, standard error, standard deviation, median, interquartile range, 95% confidence limits, results of analysis of variance, non-parametric median tests, discriminant analysis, etc., will vary as data from additional patients are added to the database or antibodies are utilized to determine concentrations of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, or any biomarker. Therefore changes in the range of concentrations of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, do not depart from the concept, spirit and scope of the invention.

[0262] Also more specifically, it is disclosed (in cross referenced U.S. Utility patent application by Goldknopf, I. L. et al. Ser. Nos. 11/507,337 and 11/503,881, U.S. Provisional Patent Applications by Goldknopf et al. Ser. Nos. 60/708,992 and 60/738,710, and referenced in Goldknopf, I. L. et al. 2006 and E. A. Sheta et al, 2006, hereby incorporated as reference) that blood serum concentrations of protein biomarkers, including an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, protein spot N3314, can be used in combination with other biomarkers for diagnosis, differential diagnosis, and screening. Consequently, the use of an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, a Transthyretin protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Complement Factor Bb protein, A Complement Factor H protein, and/or an Inter-alpha Trypsin Inhibitor Heavy Chain (H4) related protein, in conjunction with one or more additional biomarkers does not depart from the concept, spirit and scope of the invention.

[0263] It is also well recognized in the art that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

[0264] It is also well recognized in the art that there are other Non-Alzheimer's neurological disorders related to those already mentioned that are hereby included within the scope of the invention including but not limited to Mild Cognitive Impairment, Cortical basal Ganglionic Degeneration, Amyotrophic Lateral Sclerosis, and any neurological disease or disorder, injury, depression or other psychiatric condition, or any other AD-Like disorder with symptoms similar to Alzheimer's disease that results from any other cause.

Additional Tables

[0265]

TABLE 3a

Apolipoprotein E4 Protein N5302	n	Mean	±	SE	% AMC	ANOVA-P
AMC	75	81.4	±	13.43	100%	
AD	115	229.9	±	18.74	282%	<0.0001
PD	12	0.0	±	—	0%	
AD-Like + Mixed	12	57.6	±	23.20	71%	

Table 3a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Apolipoprotein E4 (spot N5302). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **3**A.

TABLE 3b

Apolipoprotein E4 Protein N5302	ROC N5302 cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			74.7%	0.66	0.020	
AD	0	55.1%				<0.0001

Table 3b: Receiver Operator Characteristics (ROC) of blood serum Apolipoprotein E4 protein N5302 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve, using biomarker N5302 cutoff concentration value of zero ppm. These results are illustrated graphically in FIG. **3**B.

TABLE 4a

Apolipoprotein E4 Protein N5302	n	Mean	±	SE	% N5302 > 0	ANOVA-P
Age Matched Controls (AMC)	75	81.4	±	13.43	31%	
AD	115	229.9	±	18.74	58%	<0.0001
Age Matched Controls (AMC) N5302 > 0	23	265.6	±	34.91		
AD N5302 > 0	67	394.7	±	26.67		<0.0001

Table 4a: Mean level (ppm)±standard error (SE) and statistical differences from AMC (ANOVA-P) of blood serum Apolipoprotein E4 protein spot N5302 in all individuals and in individuals with detectable levels of Apolipoprotein E4 protein spot N5302 in the blood serum (N5302>0). The proportion of individuals with detectable levels of Apolipoprotein E4 protein spot N5302 (N5302>0) is presented as percentage of the total number of individuals in each category. Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **4**A.

TABLE 4b

Apolipoprotein E4 Protein N5302 > 0:AD > AMC	ROC N5302 cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			50.7%	0.61	0.040	<0.0030
AD	159	64.2%				

Table 4b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Apolipoprotein E4 protein N5302 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects with detectable levels of Apolipoprotein E4 protein (N5302>0), as reflected by sensitivity, specificity, and area under the curve, using biomarker N5302 cutoff concentration value of 159 ppm. These results are illustrated graphically in FIG. **4**B.

TABLE 5a

Apolipoprotein E3 Protein N3314	n	Mean	±	SE	% AMC	ANOVA-P
Age Matched Controls (AMC)	75	1047.7	±	36.88	100%	
Alzheimer's Disease (AD)	115	656.0	±	27.01	63%	<0.0001
Parkinson's Disease (PD)	12	230.8	±	42.58	22%	
AD-Like + Mixed	12	355.4	±	48.89	34%	

Table 5a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Apolipoprotein E3 protein spot N3314. Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **5**A.

TABLE 5b

Apolipoprotein E3 Protein AD < AMC	ROC N3314 cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			64.0%	0.71	0.022	<0.0001
AD	804	64.1%				

Table 5b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Apolipoprotein E3 protein spot N3314 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve, using biomarker N3314 cutoff concentration value of AD<804 ppm. These results are illustrated graphically in FIG. **5**B.

TABLE 6

Apolipoprotein E3 Protein N3314	n	Mean	±	SE	% AMC N5302 = 0	ANOVA-P
AMC N5302 = 0	52	1094.9	±	44.10	100%	
AD N5302 = 0	48	872.9	±	42.35	80%	P < 0.0004
AMC N5302 > 0	23	940.9	±	65.92	86%	
AD N5302 > 0	67	500.6	±	30.75	46%	P < 0.0001

[0266] Table 6: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Apolipoprotein E3 protein spot N3314 when Apolipoprotein E4 protein spot N5302 is detected (N5302>0) and not detected (N5302=0) in blood serum. Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **6**A.

TABLE 7

Apolipoprotein E3 protein N3314					
	AD vs. AMC	N3314	n	Mean	SE
7a. AD < AMC (N5302 = 0) ANOVA	ANOVA P < 0.0004	AMC N5302 = 0	52	1084.8	44.10
		AD N5302 = 0	48	872.9	42.35

TABLE 7-continued

Apolipoprotein E3 protein N3314					
	AD vs. AMC	N3314	N3314⍰	Sensitivity	Specificity
ROC	ROC	AMC N5302 = 0 AD N5302 = 0	981	54.2%	53.8%
			Area	SE	ROC-P
	AD N5302 = 0	⍰ values	0.50	0.030	P < 0.0020
	AD vs. AMC	N3314	n	Mean	SE
7b. AD < AMC (N5302 > 0) ANOVA	ANOVA P < 0.0001	AMC N5302 = 0 AD N5302 = 0	23 87	940.9 502.0	55.92 ⍰.75
	AD vs. AMC	N3314	N3314⍰	Sensitivity	Specificity
ROC	ROC	AMC N5302 = 0 AD N5302 > 0	807	58.2%	88.1%
			Area	SE	ROC-P
	AD N5302 > 0	⍰ values	0.76	0.093	<0.0001
	AD vs. AD	N3314	n	Mean	SE
7c. AD (N5302 = 0) > AD (N5302 > 0) ANOVA	ANOVA P < 0.0001	AD N5302 = 0 AD N5302 > 0	48 87	877.9 533.0	42.35 33.75
	AD vs. AD	N3314	N3314⍰	Sensitivity	Specificity
ROC	ROC	AD N5302 = 0 AD N5302 > 0	651	21.6%	31.5%
			Area	SE	ROC-P
	AD N5302 > 0	⍰ values	0.78	0.080	<0.0001

⍰ indicates text missing or illegible when filed

Table 7: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Apolipoprotein E3 protein N3314 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values of the concentration of Apolipoprotein E3 protein N3314 are calculated as AD<AMC for (a) individuals when Apolipoprotein E4 protein N5302 is not detected in the blood serum (N5302=0), using biomarker N3314 cutoff concentration value of AD<981 ppm; (b) individuals when Apolipoprotein E4 protein N5302 is detected in the blood serum (N5302>0), using biomarker N3314 cutoff concentration value of AD<607 ppm; and (c) differentiation between two types of Alzheimer's disease patients with Apolipoprotein E4 protein N5302 detected (N5302>0) vs. not detected (N5302=0) in the blood serum, using biomarker N3314 cutoff concentration value of AD (N5302>0)<651 ppm. These results are illustrated graphically in FIG. **7**.

TABLE 8a

Transthyretin Protein N3307	n	Mean ± SE	% of AMC	ANOVA-P
Age Matched Controls (AMC)	75	481.9 ± 30.24	100%	
AD	115	347.0 ± 25.74	72%	<0.0001
PD	12	186.0 ± 29.83	39%	
AD-Like + Mixed	11	171.3 ± 20.76	36%	

Table 8a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Transthyretin Dimer (spot N3307). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **8**A.

TABLE 8b

Transthyretin Protein N3307	ROC N3307 < cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			60.9%	0.66	0.023	<0.0001
AD	333	60.9%				

Table 8b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Transthyretin Dimer protein spot N3307 to distinguish between Alzheimer's disease patients and age-matched normal controls (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve, using biomarker N3307 cutoff concentration value of AD<333 ppm. These results are illustrated graphically in FIG. **8**B.

TABLE 9

a: Transthyretin "Dimer" Protein N3307

Transthyretin Protein N3307	n	Mean ± SE	% of AMC	ANOVA-P
AMC N5302 = 0	52	508.3 ± 40.87	100%	
AD N5302 = 0	48	366.7 ± 23.74	72%	<0.0040
AMC N5302 > 0	23	422.3 ± 33.72	83%	
AD N5302 > 0	67	332.9 ± 40.80	65%	>0.21

b: Transthyretin "Dimer" Protein N3307

Transthyretin Protein N3307 AD < AMC	ROC N3307 < cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC N5302 = 0			55.1%	0.60	0.033	
AD N5302 = 0	352	54.9%				<0.0020
AMC N5302 > 0			65.2%	0.73	0.030	
AD N5302 > 0	308	65.2%				<0.0001

Table 9: (a) Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Transthyretin Dimer (spot N3307), when N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. (b) Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Transthyretin Dimer protein N3307 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for individuals when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, using biomarker N3307 cutoff values of AD<308 and AD<352 ppm, respectively. These results are illustrated graphically in FIGS. **9**A and B, respectively.

TABLE 10a

Complement Factor H/HS Protein N4411	n	Mean ± SE	% AMC	ANOVA-P
Age Matched Controls (AMC)	75	296.8 ± 20.85	100%	
Alzheimer's Disease (AD)	115	374.5 ± 17.66	126%	<0.0040
Parkinson's Disease (PD)	12	435.9 ± 59.48	147%	
AD-Like + Mixed	12	258.8 ± 36.75	87%	

Table 10a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Complement Factor H/Hs protein (spot N4411). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **10**A.

TABLE 10b

Complement Factor H/HS Protein N4411 AD > AMC	ROC N4411 > cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			57.3%	0.59	0.024	
AD	>261	57.4%				<0.0002

Table 10b: Summary statistics for the graph in FIG. **10***b;* Receiver Operator Characteristics (ROC) of the differences in concentration in blood serum of Complement Factor H/Hs protein spot N4411, where AD>AMC (AUC=0.59±0.024) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve, using biomarker N4411 cutoff concentration value of AD>261 ppm. These results are illustrated graphically in FIG. **10**B.

TABLE 11

a:

Complement Factor H Protein N4411	n	Mean ± SE	% of AMC	ANOVA-P
AMC N5302 = 0	52	287.3 ± 24.65	100%	
AD N5302 = 0	48	438.2 ± 29.04	153%	<0.0001
AMC N5302 > 0	23	319.3 ± 39.10	111%	
AD N5302 > 0	67	328.9 ± 21.54	114%	>0.80

b:

Complement Factor H Protein N4411 AD > ASMC	ROC N4411 > cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC N5302 = 0			62.2%	0.64	0.032	
AD N5302 = 0	270	62.5%				<0.0001
AMC N5302 > 0			49.3%	0.53	0.041	
AD N5302 > 0	273	49.3%				P > 0.22

Table 11: (a) Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Complement Factor H/Hs protein spot N4411, when N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. (b) Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Complement Factor H/Hs protein spot N4411 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for individuals when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, using biomarker N4411 cutoff concentration values of AD>273 and AD>270 ppm, respectively. These results are illustrated graphically in FIGS. **11**A and B, respectively.

TABLE 12a

Complement Factor Bb Protein N7616	n	Mean ± SE	% AMC N5302 = 0	ANOVA-P
AMC	75	276.4 ± 16.59	100%	
AD	115	298.7 ± 11.41	108%	P > 0.110
PD	12	368.2 ± 22.34	133%	
AD-Like + Mixed	12	311.0 ± 25.81	113%	

Table 12a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Complement Factor Bb protein (spot N7616). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **12**A.

TABLE 12b

Complement Factor Bb Protein N7616 AD > AMC	ROC N5302 < cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			52.4%	0.53	0.024	
AD	233	52.5%				>0.09

Table 12b: Summary statistics for the graph in FIG. **12***b*; Receiver Operator Characteristics (ROC) of the differences in concentration in blood serum of Complement Factor Bb protein (spot N7616) where AD>AMC (AUC=0.53±0.024), to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve, where AD>AMC using biomarker N7616 cutoff concentration value of AD>233 ppm. These results are illustrated graphically in FIG. **12**B.

TABLE 13

a

Complement Facto Bb Protein N7616	n	Mean ± SE	% of AMC	ANOVA-P
AMC N5302 = 0	52	262.2 ± 12.88	100%	
AD N5302 = 0	48	347.9 ± 21.14	133%	<0.0006
AMC N5302 > 0	23	308.4 ± 45.60	118%	
AD N5302 > 0	67	263.4 ± 11.85	100%	>0.17

TABLE 13-continued

Complement Facto Bb Protein N7616 AD > 0	ROC N7616 > cutoff	Sensitivity	Specificity	Area	SE	ROC-P
			b			
AMC N5302 = 0			55.8%	0.59	0.033	
AD N5302 = 0	237	55.6%				<0.0040
AMC N5302 > 0			49.3%	0.48	0.039	
AD N5302 > 0	229	50.7%				P > 0.6

Table 13: (a) Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Complement Factor Bb protein biomarker spot N7616, when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. (b) Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Complement Factor Bb protein biomarker spot N7616 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for individuals when N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, where AD>AMC using biomarker N7616 cutoff concentration values of AD>229 and AD>237 ppm, respectively. These results are illustrated graphically in FIGS. **13**A and B, respectively.

TABLE 14

	Complement C3c1 Phosphoprotein N7310 Acquired Auto-Immune Inflammation					
	N7310	n	Mean	Median	SE	Mean % AMC
14a:	Age Matched Control	75	315.8	241.7	10.57	100%
	AD	115	884.5	330.0	80.81	280%
	PD	12	1380.7	1230.0	190.20	443%
	AD-Like and Mixed	12	300.2	213.3	72.23	07%

TABLE 14-continued

	Complement C3dg Protein N1511 Innate + Acquired Auto-Immune Inflammation					
	N1511	n	Mean	Median	SE	Mean % AMC
14b:	Age Matched Control	75	400.5	74.1	8.65	100%
	AD	115	457.9	190.1	31.51	416%
	PD	12	792.3	642.7	75.30	723%
	AD-Like and Mixed	12	400.7	250.2	50.23	371%
	Complement C3c2a Protein N9311 Innate Immune Inflammation					
	N9311	n	Mean	Median	SE	Mean % AMC
14c:	Age Matched Control	75	305.7	250.1	12.03	100%
	AD	115	580.5	258.8	35.07	100%
	PD	12	728.1	635.3	5.37	238%
	AD-Like and Mixed	12	802.1	437.8	92.01	197%
	Complement C3Sum = N7310 + N9311 + N1511 Innate + Acquired Auto-Immune Inflammation					
	C3Sum	n	Mean	Median	SE	Mean % AMC
14d:	Age Matched Control	75	731.1	843.4	29.19	100%
	AD	115	1922.0	929.6	112.72	283%
	PD	12	2920.2	3220.0	259.80	399%
	AD-Like and Mixed	12	1314.0	1155.0	133.23	180%

Table 14: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and Median (50^{th} percentile) of blood serum a) Complement C3c1 phosphoprotein (spot N7310); b) Complement C3dg protein spot N1511; c) Complement C3c2a protein (spot N9311); Complement C3Sum (N7310+N1511+N9311). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **14**.

TABLE 15

	N1511 ROC	N1511 > cutoff	Sensitivity	Specificity	ROC-P	ANOVA-P
15a	AD > AMC	>105	60.9%	60.9%	<0.0001	<0.0001

TABLE 15-continued

	N7310 ROC	N7310 > cutoff	Sensitivity	Specificity	ROC-P	ANOVA-P
15b	AD > AMC	>273	65.9%	55.5%	<0.0001	<0.0001
	N9311 ROC	N9311 > cutoff	Sensitivity	Specificity	ROC-P	ANOVA-P
15c	AD > AMC	>273	53.9%	53.8%	<0.0001	<0.0001
	C3Sum ROC	C3Sum > cutoff	Sensitivity	Specificity	ROC-P	ANOVA-P
15d	AD > AMC	>710	55.1%	55.1%	<0.0001	<0.0001

Table 15: Receiver Operator Characteristics (ROC) of the differences in blood serum concentrations of a) Complement C3dg protein (spot N1511); b) Complement C3c1 phosphoprotein (spot N7310); Complement C3c2a protein (spot N9311); and C3Sum (N1511+N7310+N9311) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and statistical significance, where AD>AMC using cutoff concentration values for each at Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of AD>105, AD>273, AD>272, and AD>710 ppm, respectively. These results are illustrated graphically in FIG. **15**.

TABLE 16

16a

Summary	Complement C3c1 Phosphoprotein N7310 Acquired AutoImmune Inflammation						Complement C3c2a Protein N9311 Innate Inflammation					
Statistics	N7310	n	Mean	Median	SE	% Change	N9311	n	Mean	Median	SE	% Change
AMC ⍰ =⍰	ANOVA	62	332.6	270.3	2100	303%	ANOVA	62	335.1	278.2	16.11	128%
AD ⍰ =⍰	P < 0.0001	18	⍰	⍰	110.16		P < 0.0001	18	⍰	298.1	50.15	
AMC ⍰ 0	ANOVA	23	⍰	180.2	403.7	⍰%	ANOVA	23	251.9	225.1	10.34	206%
AD ⍰ 0	P < 0.0001	67	743.1	296.3	666.7		P < 0.0001	61	540.0	296.7	41.43	
AD ⍰ = 0	ANOVA	18	⍰	⍰	110.16	70%	ANOVA	18	53.1	298.1	53.15	85%
AD ⍰ 0	P < 0.0001	67	745.1	296.3	65.57		P > 0.11	67	540.0	⍰	41.43	

16b

Summary	Complement C3dg Protein N1511 Innate + Acquired AutoImmune Inflammation						Complement C3Sum = N1511 + N7310 + N9311 Innate + Acquired AutoImmune Inflammation					
Statistics	N1511	n	Mean	Median	SE	% Change	C3Sum	n	Mean	Median	SE	% Change
AMC N5302 = 0	ANOVA	62	105.8	69.4	103.0	511%	ANOVA	62	753.4	604.8	30.59	295%
AD N5302 = 0	P < 0.0001	18	510.3	210.3	95.53		P < 0.0001	18	2253.3	⍰	203.07	
AMC N5302 > 0	ANOVA	23	110.0	199	1590	300%	ANOVA	23	⍰	⍰	56.11	266%
AD N5302 > 0	P < 0.0001	67	⍰	167.6	36.82		P < 0.0001	61	⍰	116.0	136.30	
AD N5302 = 0	ANOVA	18	540.3	⍰	55.98	74%	ANOVA	18	⍰	⍰	203.07	78%
AD N5302 > 0	P < 0.0001	67	⍰	167.6	36.82		P < 0.0001	67	1687.0	715.0	136.38	

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Table 16: Mean level (ppm)±standard error (SE), Median (50^{th} percentile) and percent change in blood serum levels of a) Complement C3c1 phosphoprotein (spot N7310), Complement C3c2a protein (spot N9311), and b) Complement C3dg protein spot N1511, and Complement C3Sum (N7310+N1511+N9311) when Apolipoprotein E4 protein N5302 is not detected (N5302=0) and detected (N5302>0) in the blood serum. These results are illustrated graphically in FIG. **16**.

TABLE 17

17a

Receiver Operator Characteristics		Complement C3c1 Phosphoprotein N7310 Acquired Auto-Immune Inflammation				Complement C3c2a Protein N9311 Innate Inflammation				
		N7310 cutoff	Sensitivity	Specificity	ROC-P	N9311 cutoff	Sensitivity	Specificity	ROC-P	Class
AD vs. AMC at Sensitivity ⍰ Specificity	AMC ⍰			⍰	⍰			⍰	⍰	Not AD
	⍰	⍰	⍰			⍰	⍰			AD
AD vs. AMC at Sensitivity Specificity	AMC ⍰			⍰	⍰			⍰	⍰	Not AD
	AD ⍰	⍰	⍰			⍰	⍰			AD
AD vs. AD at Sensitivity Specificity	AD ⍰	⍰	⍰		⍰	⍰	⍰		⍰	AD
	AD ⍰			⍰				⍰		AD

17b

Receiver Operator Characteristics (ROC)		Complement C3dg Protein N1511 Innate + Acquired Immune Inflammation				Complement C3Sum = N1511 + N7310 + N9311 Innate + Acquired Immune Inflammation				
		N1511 cutoff	Sensitivity	Specificity	ROC-P	C3Sum cutoff	Sensitivity	Specificity	ROC-P	Class
AD vs. AMC at Sensitivity ⍰ Specifivity	AMC N5302 = 0			62.8%	<0.0001			56.4%	<0.0001	Not AD
	AD N5302 = 0	>107	63.2%			>743	56.3%			AD
AD vs. AMC at Sensitivity ⍰ Specifivity	AMC N5302 > 0			58.0%	<0.0001			58.0%	<0.0001	Not AD
	AD N5302 > 0	>106	58.2%			>635	58.2%			AD
AD vs. AD at Sensitivity ⍰ Specifivity	AD N5302 = 0	>196	51.4%		>0.110	>769	56.3%		<0.0009	AD
	AD N5302 > 0			51.2%				56.3%		Not AD

⍰ indicates text missing or illegible when filed

Table 17: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of a) Complement C3c1 phosphoprotein (spot N7310), Complement C3c2a protein (spot N9311), and b) Complement C3dg protein (spot N1511), and C3Sum (N1511+N7310+N9311) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects and between two Alzheimer's disease patients, when Apolipoprotein E4 protein spot N5302 was not detected (N5302=0) and detected (N5302>0), as reflected by sensitivity, specificity, and statistical significance, using a characteristic cutoff concentration value for each biomarker. These results are illustrated graphically in FIG. **17**.

TABLE 18a

Haptoglobin HP-1 Total Proteins N1514 + N2401 + N2407 + N3409	n	Mean ± SE	% AMC	ANOVA-P
AMC	75	29434.0 ± 703.64	100%	
AD	115	32898.4 ± 618.74	112%	0.0028
PD	12	29387.4 ± 1574.31	100%	
AD-Like + Mixed	12	32316.8 ± 2522.66	110%	

Table 18a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical significance of blood serum Total Haptoglobin Hp-1 proteins (Sum of spots N1514+N2401+N2407+N3409). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **22**A.

TABLE 18b

Haptoglobin HP-1 Total Proteins N1514 + N2401 + N2407 + N3409	ROC Hp-1 Total > cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			56.0%	0.59	0.024	
AD	30136	56.2%				<0.0001

Table 18b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of

[0267] Total Haptoglobin Hp-1 proteins (Sum of spots N1514+N2401+N2407+N3409) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, area under the curve of ROC and statistical significance, where AD>AMC using cutoff value at AD>30136 ppm. These results are illustrated graphically in FIG. **22**B.

TABLE 19a

Total HP-1 Proteins N1514 + N2401 + N2407 + N3409 AD > AMC	ROC Total HP-1 < cutoff	Sensi-tivity	Speci-ficity	Area	SE	ROC-P
AMC N5302 = 0			64.7%	0.68	0.031	
AD N5302 = 0	30216	64.6%				<0.0001
AMC N5302 > 0			44.9%	0.47	0.040	
AD N5302 > 0	31768	44.8%				>0.14

Table 19a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical differences from AMC (ANOVA-P) of blood serum total Haptoglobin Hp-1 proteins (Sum of spots N1514+N2401+N2407+N3409), when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. These results are illustrated graphically in FIG. **25**A.

TABLE 19b

Total HP-1 Proteins N1514 + N2401 + N2407 + N3409 AD > AMC	ROC Total HP-1 < cutoff	Sensi-tivity	Specifi-city	Area	SE	ROC-P
AMC N5302 = 0			64.7%	0.68	0.031	
AD N5302 = 0	30216	64.6%				<0.0001
AMC N5302 > 0			44.9%	0.47	0.040	
AD N5302 > 0	31768	44.8%				>0.14

Table 19b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of total Haptoglobin Hp-1 proteins (Sum of spots N1514+N2401+N2407+N3409) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for each group when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, where AD>AMC using cutoff values of AD>31768 and AD>30216 ppm, respectively. These results are illustrated graphically in FIG. **25**B.

TABLE 20a

Inter-alpha-trypsin Inhibitor Heavy Chain (H4) Related 35 KD Protein N2307	n	Mean ± SE	% AMC	ANOVA-P
AMC	75	241.0 ± 13.74	100%	
AD	115	410.2 ± 20.41	170%	<0.0001
PD	12	408.6 ± 54.66	170%	
AD-Like + Mixed	12	327.6 ± 51.07	136%	

Table 20a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical significance of blood serum Inter-alpha-trypsin inhibitor heavy chain (H4) related 35 KD protein (spot N2307). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **26**A.

TABLE 20b

Inter-alpha-trypsin Inhibitor Heavy Chain (H4) Related 35 KD Protein N2307 AD > AMC	ROC N2307 > cutoff	Sensi-tivity	Speci-ficity	Area	SE	ROC-P
AMC			58.2%	0.62	0.023	
AD	210	58.0%				<0.0001

Table 20b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Inter-alpha-trypsin inhibitor heavy chain (H4) related 35 KD protein (spot N2307) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, area under the curve of ROC and statistical significance, where AD>AMC using cutoff concentration value at AD>210 ppm. These results are illustrated graphically in FIG. **26**B.

TABLE 21a

Inter-alpha-trypsin inhibitor heavy chain (H4) Related 35 KD Protein N2307	n	Mean ± SE	% AMC N5302 = 0	ANOVA
AMC N5302 = 0	52	215.9 ± 14.49	100%	
AD N5302 = 0	48	457.6 ± 36.34	212%	P < 0.0001
AMC N5302 > 0	23	297.9 ± 29.62	138%	
AD N5302 > 0	67	376.1 ± 23.23	174%	P > 0.06

Table 21a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Inter-alpha-trypsin inhibitor heavy chain (H4) related 35 KD protein (spot N2307), when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. These results are illustrated graphically in FIG. **27**A.

TABLE 21b

Inter-alpha-trypsin inhibitor heavy chain (H4) Related 35 KD Protein N2307 AD > AMC	ROC N2307 < cutoff	Sensi-tivity	Speci-ficity	Area	SE	ROC-P
AMC N5302 = 0			61.5%	0.68	0.031	
AD N5302 = 0	211	61.8%				<0.0001
AMC N5302 > 0			50.7%	0.54	0.038	
AD N5302 > 0	224	50.7%				>0.14

Table 21b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Inter-alpha-trypsin inhibitor heavy chain (H4) related 35 KD protein spot N2307 to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for each group when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, where AD>AMC using cutoff concentration values of AD>224 and AD>211 ppm, respectively. These results are illustrated graphically in FIG. **27**B.

TABLE 22a

Immunoglobulin Light Chain Protein N6224	n	Mean ± SE	% AMC	ANOVA-P
AMC	75	461.1 ± 16.74	100%	
AD	115	369.4 ± 15.35	80%	<0.0002
PD	12	336.1 ± 24.24	73%	
AD-Like + Mixed	12	329.1 ± 39.04	71%	

Table 22a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical significance of blood serum Immunoglobulin light chain protein (spot N6224). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **29**A.

TABLE 22b

Immunoglobulin Light Chain Protein N6224 AD < AMC	ROC N6224 < cutoff	Sensi-tivity	Speci-ficity	Area	SE	ROC-P
AMC			59.6%	0.64	0.023	
AD	368	59.7%				<0.0001

Table 22b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Immunoglobulin light chain protein (spot N6224) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, area under the curve of ROC and statistical significance, where AD<AMC using cutoff concentration value at AD<368 ppm. These results are illustrated graphically in FIG. **29**B.

TABLE 23a

Immunoglobulin Light Chain Protein N6224	n	Mean ± SE	% AMC N5302 = 0	ANOVA
AMC N5302 = 0	52	452.4 ± 19.81	100%	
AD N5302 = 0	48	391.2 ± 30.15	86%	P > 0.08
AMC N5302 > 0	23	480.7 ± 31.27	106%	
AD N5302 > 0	67	353.7 ± 15.06	78%	P < 0.0007

Table 23a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Immunoglobulin light chain protein (spot N6224), when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. These results are illustrated graphically in FIG. **30**B.

TABLE 23b

Immunoglobulin Light Chain Protein N6224 AD < AMC	ROC N6221 < cutoff	Sensi-tivity	Speci-ficity	Area	SE	ROC-P
AMC N5302 = 0			57.1%	0.62	0.032	
AD N5302 = 0	368	56.9%				<0.0003
AMC N5302 > 0			62.3%	0.68	0.036	
AD N5302 > 0	378	62.7%				<0.0001

Table 23b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Immunoglobulin light chain protein (spot N6224) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for each group when N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, where AD<AMC using cutoff values of AD<378 and AD<368 ppm, respectively. These results are illustrated graphically in FIG. **30**B.

TABLE 24a

Apolipoprotein A-IV Protein N2502	n	Mean ± SE	% AMC	ANOVA-P
AMC	75	2915.6 ± 115.15	100%	
AD	115	2341.8 ± 74.61	80%	<0.0001
PD	12	1737.8 ± 180.58	60%	
AD-Like + Mixed	12	1934.2 ± 191.21	66%	

Table 24a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal controls (AMC) and statistical significance of blood serum Apolipoprotein A-IV protein (spot N2502). Each sample (n) was run in triplicate on three separate 2D gels. These results are illustrated graphically in FIG. **31**B.

TABLE 24b

Apolipoprotein A-IV Protein N2502	ROC N2502 < cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC			59.1%	0.64	0.023	
AD	2465	59.1%				<0.0001

Table 24b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of Apolipoprotein A-IV protein (spot N2502) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, area under the curve of ROC and statistical significance, where AD<AMC using N2502 cutoff value at AD<2465 ppm. These results are illustrated graphically in FIG. **31**B.

TABLE 25a

Apolipoprotein A-IV Protein N2502	n	Mean ± SE	% AMC N5302 = 0	ANOVA
AMC N5302 = 0	52	2993.5 ± 155.71	100%	
AD N5302 = 0	48	2339.1 ± 131.43	78%	P < 0.0003
AMC N5302 > 0	23	2739.7 ± 129.70	92%	
AD N5302 > 0	67	2343.8 ± 87.10	78%	P < 0.03

Table 25a: Mean level (ppm)±standard error (SE), percent change from the mean level of age-match normal control (AMC) and statistical differences from AMC (ANOVA-P) of blood serum Apolipoprotein A-IV protein (spot N2502), when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum. These results are illustrated graphically in FIG. **32**A.

TABLE 25b

Apolipoprotein A-IV Protein N2502 AD < AMC	ROC N2502 < cutoff	Sensitivity	Specificity	Area	SE	ROC-P
AMC N5302 = 0			58.3%	0.65	0.032	
AD N5302 = 0	2412	58.3%				<0.0001
AMC N5302 > 0			62.3%	0.64	0.039	
AD N5302 > 0	2588	62.2%				<0.0003

Table 25b: Receiver Operator Characteristics (ROC) of the difference in blood serum concentrations of

[0268] Apolipoprotein A-IV protein (spot N2502) to distinguish between Alzheimer's disease patients and age-matched normal control (AMC) subjects, as reflected by sensitivity, specificity, and area under the curve for the ROC. Values are calculated for each group when Apolipoprotein E4 protein N5302 is detected (N5302>0) and not detected (N5302=0) in the blood serum, where AD<AMC using cutoff concentration values of AD<2588 and AD2412 ppm, respectively. These results are illustrated graphically in FIG. **32**B.

TABLE 26

Linear Discriminant Function	Biomarkers Employed in Discriminant Function	Sensitivity	Specificity
All Samples	N5302 N3314 N3307 N4411 N7616 HaptogI N7310 N9311 N1511 N2307	69.6%	84.4%
Combined	N2502 N6224		
Samples Separated	N5302 N3314 N3307 N4411 N7616 HaptogI N7310 N9311 N1511 N2307	82.3%	82.7%
N5302 = 0 + N5302 > 0	N2502 N6224		

Table 26: Enhanced sensitivity obtained by applying multivariate linear discriminant biostatistics to the blood serum concentrations of the listed protein biomarkers. The first approach employs comparing Alzheimer's disease patients and age-matched control using the listed biomarkers without sorting the compared groups. The second approach employs the separation of both Alzheimer's disease patients and age-matched control subjects into two categories based on the detection or lack of detection of Apolipoprotein E4 protein N5302 in their blood serum. A multivariate biostatistical analysis is applied to each of the 2 groups, employing all the biomarkers listed (N3314, N3317, N4411; N7616, HP-1 total [N1514+N2401+N2407+N3409], N7310, N9311, N1511, N2307, N2502, and N6224), followed by summing the separate results of the 2 multivariate biostatistical analysis of the sorted categories. As shown, this second approach provides substantial improvement in diagnostic capability over the first, non-sorted approach. These results are illustrated graphically in FIG. **33**.

TABLE 27

Mechanism of Neuronal	Alzheimer's disease		Amyotrophic lateral sclerosis (ALS)§	
Degeneration	Apo E4 > 0	Apo E4 = 0	Familial	Sporadic
Neuronal Oxidative Stress and Apoptosis	Primary	Inhibited	Primary	Inhibited
Autoimmune/ Innate Inflammation	Secondary	Primary	Secondary	Primary

§From references 17, 18.

Table 27: Observed similarity in the mechanism of neuronal degeneration in Alzheimer's disease and Amyotrophic lateral sclerosis patients, drawn from the identities, functions and observed differences in blood serum concentration of the listed biomarkers.

TABLE 28¥

	Summary multivariate statistics using 34 biomarkers			Summary multivariate statistics using 24 biomarkers		
Statistical Test	AD (n = 22)	PD (n = 29)	ALS (n = 136)	AD (n = 44)	PD (n = 24)	Normal
Linear	91%	79%	89%	86%	92%	94%

¥From reference 19

Table 28ᵛ: Multivariate linear discriminant analysis as indicated by percent sensitivity of classification of each disease in mixture of population, using 34 and step disc-selected 24 serum biomarkers.

TABLE 29

The examples illustrate how the invention:

Provides a relational perspective from the patients to functional, pre-clinical, and clinical studies of genomic and proteomic biomarkers
Enables differential diagnostic and disease specific mechanism discrimination between
- Similar diseases, e.g. AD vs. ALS vs. PD; AD vs. AD-Like vs. Normal
- Sporadic and familial disease subcategories, e.g. Apo E4 (+) AD vs. Apo E4 (−) AD; and sALS vs. fALS
- Disease mechanisms, e.g. oxidative stress, apoptosis, and autoimmune inflammatory mechanisms of neuronal degeneration.

Provides the type of information that can be employed in the monitoring of patients for:
- Potential drug response
- Disease severity and progression
- Potential new drug targets

Will ultimately lead to personalized medicine

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 22

<210> SEQ ID NO 1
<211> LENGTH: 317
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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Met Lys Val Leu Trp Ala Ala Leu Leu Val Thr Phe Leu Ala Gly Cys
1               5                   10                  15

Gln Ala Lys Val Glu Gln Ala Val Glu Thr Glu Pro Glu Pro Glu Leu
            20                  25                  30

Arg Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu
        35                  40                  45

Gly Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln
    50                  55                  60

Val Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala
65                  70                  75                  80

Leu Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu
                85                  90                  95

Glu Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser
            100                 105                 110

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

Val Arg Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu
    130                 135                 140

Gly Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg
145                 150                 155                 160

Lys Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg
                165                 170                 175

Leu Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu
            180                 185                 190
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Ser Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val
        195                 200                 205

Arg Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg
    210                 215                 220

Ala Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly
225                 230                 235                 240

Ser Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu
                245                 250                 255

Val Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala
            260                 265                 270

Glu Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu
        275                 280                 285

Asp Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala
    290                 295                 300

Val Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
305                 310                 315


<210> SEQ ID NO 2
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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

Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu Gly
            20                  25                  30

Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln Val
        35                  40                  45

Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala Leu
    50                  55                  60

Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu Glu
65                  70                  75                  80

Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser Lys
                85                  90                  95

Glu Leu Gln Ala Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp Val
            100                 105                 110

Arg Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu Gly
        115                 120                 125

Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg Lys
    130                 135                 140

Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg Leu
145                 150                 155                 160

Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu Ser
                165                 170                 175

Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val Arg
            180                 185                 190

Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg Ala
        195                 200                 205

Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly Ser
    210                 215                 220

Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu Val
225                 230                 235                 240
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Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala Glu
                245                 250                 255

Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu Asp
            260                 265                 270

Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala Val
        275                 280                 285

Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
    290                 295                 300


<210> SEQ ID NO 3
<211> LENGTH: 317
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Lys Val Leu Trp Ala Ala Leu Leu Val Thr Phe Leu Ala Gly Cys
1               5                   10                  15

Gln Ala Lys Val Glu Gln Ala Val Glu Thr Glu Pro Glu Pro Glu Leu
            20                  25                  30

Arg Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu
        35                  40                  45

Gly Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln
    50                  55                  60

Val Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala
65                  70                  75                  80

Leu Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu
                85                  90                  95

Glu Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser
            100                 105                 110

Lys Glu Leu Gln Thr Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp
        115                 120                 125

Val Cys Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu
    130                 135                 140

Gly Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg
145                 150                 155                 160

Lys Leu Arg Lys Arg Leu Leu Arg Asp Pro Asp Asp Leu Gln Lys Arg
                165                 170                 175

Leu Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu
            180                 185                 190

Ser Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val
        195                 200                 205

Arg Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg
    210                 215                 220

Ala Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly
225                 230                 235                 240

Ser Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu
                245                 250                 255

Val Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala
            260                 265                 270

Glu Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu
        275                 280                 285

Asp Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala
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    290                 295                 300

Val Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
305                 310                 315


<210> SEQ ID NO 4
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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

Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu Gly
            20                  25                  30

Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln Val
        35                  40                  45

Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala Leu
    50                  55                  60

Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu Glu
65                  70                  75                  80

Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser Lys
                85                  90                  95

Glu Leu Gln Thr Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp Val
            100                 105                 110

Cys Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu Gly
        115                 120                 125

Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg Lys
    130                 135                 140

Leu Arg Lys Arg Leu Leu Arg Asp Pro Asp Asp Leu Gln Lys Arg Leu
145                 150                 155                 160

Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu Ser
                165                 170                 175

Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val Arg
            180                 185                 190

Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg Ala
        195                 200                 205

Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly Ser
    210                 215                 220

Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu Val
225                 230                 235                 240

Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala Glu
                245                 250                 255

Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu Asp
            260                 265                 270

Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala Val
        275                 280                 285

Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
    290                 295                 300


<210> SEQ ID NO 5
<211> LENGTH: 147
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 5

Met Ala Ser His Arg Leu Leu Leu Leu Cys Leu Ala Gly Leu Val Phe
1 5 10 15

Val Ser Glu Ala Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys Pro Leu
20 25 30

Met Val Lys Val Leu Asp Ala Val Arg Gly Ser Pro Ala Ile Asn Val
35 40 45

Ala Val His Val Phe Arg Lys Ala Ala Asp Asp Thr Trp Glu Pro Phe
50 55 60

Ala Ser Gly Lys Thr Ser Glu Ser Gly Glu Leu His Gly Leu Thr Thr
65 70 75 80

Glu Glu Glu Phe Val Glu Gly Ile Tyr Lys Val Glu Ile Asp Thr Lys
85 90 95

Ser Tyr Trp Lys Ala Leu Gly Ile Ser Pro Phe His Glu His Ala Glu
100 105 110

Val Val Phe Thr Ala Asn Asp Ser Gly Pro Arg Arg Tyr Thr Ile Ala
115 120 125

Ala Leu Leu Ser Pro Tyr Ser Tyr Ser Thr Thr Ala Val Val Thr Asn
130 135 140

Pro Lys Glu
145

<210> SEQ ID NO 6
<211> LENGTH: 1663
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Gly Pro Thr Ser Gly Pro Ser Leu Leu Leu Leu Leu Thr His
1 5 10 15

Leu Pro Leu Ala Leu Gly Ser Pro Met Tyr Ser Ile Ile Thr Pro Asn
20 25 30

Ile Leu Arg Leu Glu Ser Glu Glu Thr Met Val Leu Glu Ala His Asp
35 40 45

Ala Gln Gly Asp Val Pro Val Thr Val Thr Val His Asp Phe Pro Gly
50 55 60

Lys Lys Leu Val Leu Ser Ser Glu Lys Thr Val Leu Thr Pro Ala Thr
65 70 75 80

Asn His Met Gly Asn Val Thr Phe Thr Ile Pro Ala Asn Arg Glu Phe
85 90 95

Lys Ser Glu Lys Gly Arg Asn Lys Phe Val Thr Val Gln Ala Thr Phe
100 105 110

Gly Thr Gln Val Val Glu Lys Val Val Leu Val Ser Leu Gln Ser Gly
115 120 125

Tyr Leu Phe Ile Gln Thr Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr
130 135 140

Val Leu Tyr Arg Ile Phe Thr Val Asn His Lys Leu Leu Pro Val Gly
145 150 155 160

Arg Thr Val Met Val Asn Ile Glu Asn Pro Glu Gly Ile Pro Val Lys
165 170 175

Gln Asp Ser Leu Ser Ser Gln Asn Gln Leu Gly Val Leu Pro Leu Ser
180 185 190

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Trp Asp Ile Pro Glu Leu Val Asn Met Gly Gln Trp Lys Ile Arg Ala
        195                 200                 205

Tyr Tyr Glu Asn Ser Pro Gln Gln Val Phe Ser Thr Glu Phe Glu Val
    210                 215                 220

Lys Glu Tyr Val Leu Pro Ser Phe Glu Val Ile Val Glu Pro Thr Glu
225                 230                 235                 240

Lys Phe Tyr Tyr Ile Tyr Asn Glu Lys Gly Leu Glu Val Thr Ile Thr
                245                 250                 255

Ala Arg Phe Leu Tyr Gly Lys Lys Val Glu Gly Thr Ala Phe Val Ile
            260                 265                 270

Phe Gly Ile Gln Asp Gly Glu Gln Arg Ile Ser Leu Pro Glu Ser Leu
        275                 280                 285

Lys Arg Ile Pro Ile Glu Asp Gly Ser Gly Glu Val Val Leu Ser Arg
    290                 295                 300

Lys Val Leu Leu Asp Gly Val Gln Asn Pro Arg Ala Glu Asp Leu Val
305                 310                 315                 320

Gly Lys Ser Leu Tyr Val Ser Ala Thr Val Ile Leu His Ser Gly Ser
                325                 330                 335

Asp Met Val Gln Ala Glu Arg Ser Gly Ile Pro Ile Val Thr Ser Pro
            340                 345                 350

Tyr Gln Ile His Phe Thr Lys Thr Pro Lys Tyr Phe Lys Pro Gly Met
        355                 360                 365

Pro Phe Asp Leu Met Val Phe Val Thr Asn Pro Asp Gly Ser Pro Ala
    370                 375                 380

Tyr Arg Val Pro Val Ala Val Gln Gly Glu Asp Thr Val Gln Ser Leu
385                 390                 395                 400

Thr Gln Gly Asp Gly Val Ala Lys Leu Ser Ile Asn Thr His Pro Ser
                405                 410                 415

Gln Lys Pro Leu Ser Ile Thr Val Arg Thr Lys Lys Gln Glu Leu Ser
            420                 425                 430

Glu Ala Glu Gln Ala Thr Arg Thr Met Gln Ala Leu Pro Tyr Ser Thr
        435                 440                 445

Val Gly Asn Ser Asn Asn Tyr Leu His Leu Ser Val Leu Arg Thr Glu
    450                 455                 460

Leu Arg Pro Gly Glu Thr Leu Asn Val Asn Phe Leu Leu Arg Met Asp
465                 470                 475                 480

Arg Ala His Glu Ala Lys Ile Arg Tyr Tyr Thr Tyr Leu Ile Met Asn
                485                 490                 495

Lys Gly Arg Leu Leu Lys Ala Gly Arg Gln Val Arg Glu Pro Gly Gln
            500                 505                 510

Asp Leu Val Val Leu Pro Leu Ser Ile Thr Thr Asp Phe Ile Pro Ser
        515                 520                 525

Phe Arg Leu Val Ala Tyr Tyr Thr Leu Ile Gly Ala Ser Gly Gln Arg
    530                 535                 540

Glu Val Val Ala Asp Ser Val Trp Val Asp Val Lys Asp Ser Cys Val
545                 550                 555                 560

Gly Ser Leu Val Val Lys Ser Gly Gln Ser Glu Asp Arg Gln Pro Val
                565                 570                 575

Pro Gly Gln Gln Met Thr Leu Lys Ile Glu Gly Asp His Gly Ala Arg
            580                 585                 590

Val Val Leu Val Ala Val Asp Lys Gly Val Phe Val Leu Asn Lys Lys
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        595                 600                 605

Asn Lys Leu Thr Gln Ser Lys Ile Trp Asp Val Val Glu Lys Ala Asp
    610                 615                 620

Ile Gly Cys Thr Pro Gly Ser Gly Lys Asp Tyr Ala Gly Val Phe Ser
625                 630                 635                 640

Asp Ala Gly Leu Thr Phe Thr Ser Ser Ser Gly Gln Gln Thr Ala Gln
                645                 650                 655

Arg Ala Glu Leu Gln Cys Pro Gln Pro Ala Ala Arg Arg Arg Arg Ser
            660                 665                 670

Val Gln Leu Thr Glu Lys Arg Met Asp Lys Val Gly Lys Tyr Pro Lys
        675                 680                 685

Glu Leu Arg Lys Cys Cys Glu Asp Gly Met Arg Glu Asn Pro Met Arg
    690                 695                 700

Phe Ser Cys Gln Arg Arg Thr Arg Phe Ile Ser Leu Gly Glu Ala Cys
705                 710                 715                 720

Lys Lys Val Phe Leu Asp Cys Cys Asn Tyr Ile Thr Glu Leu Arg Arg
                725                 730                 735

Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg Ser Asn Leu Asp
            740                 745                 750

Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg Ser Glu Phe Pro
        755                 760                 765

Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu Pro Pro Lys Asn
    770                 775                 780

Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys Asp Ser Ile Thr
785                 790                 795                 800

Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys Lys Gly Ile Cys
                805                 810                 815

Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp Phe Phe Ile Asp
            820                 825                 830

Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln Val Glu Ile Arg
        835                 840                 845

Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu Lys Val Arg Val
    850                 855                 860

Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala Thr Thr Lys Arg
865                 870                 875                 880

Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser Ser Leu Ser Val
                885                 890                 895

Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln Glu Val Glu Val
            900                 905                 910

Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly Val Arg Lys Ser
        915                 920                 925

Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys Thr Val Ala Val
    930                 935                 940

Arg Thr Leu Asp Pro Glu Arg Leu Gly Arg Glu Gly Val Gln Lys Glu
945                 950                 955                 960

Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln Val Pro Asp Thr Glu Ser
                965                 970                 975

Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro Val Ala Gln Met Thr Glu
            980                 985                 990

Asp Ala Val Asp Ala Glu Arg Leu  Lys His Leu Ile Val  Thr Pro Ser
        995                 1000                1005
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Gly Cys  Gly Glu Gln Asn Met  Ile Gly Met Thr Pro  Thr Val Ile
    1010                1015                1020

Ala Val  His Tyr Leu Asp Glu  Thr Glu Gln Trp Glu  Lys Phe Gly
    1025                1030                1035

Leu Glu  Lys Arg Gln Gly Ala  Leu Glu Leu Ile Lys  Lys Gly Tyr
    1040                1045                1050

Thr Gln  Gln Leu Ala Phe Arg  Gln Pro Ser Ser Ala  Phe Ala Ala
    1055                1060                1065

Phe Val  Lys Arg Ala Pro Ser  Thr Trp Leu Thr Ala  Tyr Val Val
    1070                1075                1080

Lys Val  Phe Ser Leu Ala Val  Asn Leu Ile Ala Ile  Asp Ser Gln
    1085                1090                1095

Val Leu  Cys Gly Ala Val Lys  Trp Leu Ile Leu Glu  Lys Gln Lys
    1100                1105                1110

Pro Asp  Gly Val Phe Gln Glu  Asp Ala Pro Val Ile  His Gln Glu
    1115                1120                1125

Met Ile  Gly Gly Leu Arg Asn  Asn Asn Glu Lys Asp  Met Ala Leu
    1130                1135                1140

Thr Ala  Phe Val Leu Ile Ser  Leu Gln Glu Ala Lys  Asp Ile Cys
    1145                1150                1155

Glu Glu  Gln Val Asn Ser Leu  Pro Gly Ser Ile Thr  Lys Ala Gly
    1160                1165                1170

Asp Phe  Leu Glu Ala Asn Tyr  Met Asn Leu Gln Arg  Ser Tyr Thr
    1175                1180                1185

Val Ala  Ile Ala Gly Tyr Ala  Leu Ala Gln Met Gly  Arg Leu Lys
    1190                1195                1200

Gly Pro  Leu Leu Asn Lys Phe  Leu Thr Thr Ala Lys  Asp Lys Asn
    1205                1210                1215

Arg Trp  Glu Asp Pro Gly Lys  Gln Leu Tyr Asn Val  Glu Ala Thr
    1220                1225                1230

Ser Tyr  Ala Leu Leu Ala Leu  Leu Gln Leu Lys Asp  Phe Asp Phe
    1235                1240                1245

Val Pro  Pro Val Val Arg Trp  Leu Asn Glu Gln Arg  Tyr Tyr Gly
    1250                1255                1260

Gly Gly  Tyr Gly Ser Thr Gln  Ala Thr Phe Met Val  Phe Gln Ala
    1265                1270                1275

Leu Ala  Gln Tyr Gln Lys Asp  Ala Pro Asp His Gln  Glu Leu Asn
    1280                1285                1290

Leu Asp  Val Ser Leu Gln Leu  Pro Ser Arg Ser Ser  Lys Ile Thr
    1295                1300                1305

His Arg  Ile His Trp Glu Ser  Ala Ser Leu Leu Arg  Ser Glu Glu
    1310                1315                1320

Thr Lys  Glu Asn Glu Gly Phe  Thr Val Thr Ala Glu  Gly Lys Gly
    1325                1330                1335

Gln Gly  Thr Leu Ser Val Val  Thr Met Tyr His Ala  Lys Ala Lys
    1340                1345                1350

Asp Gln  Leu Thr Cys Asn Lys  Phe Asp Leu Lys Val  Thr Ile Lys
    1355                1360                1365

Pro Ala  Pro Glu Thr Glu Lys  Arg Pro Gln Asp Ala  Lys Asn Thr
    1370                1375                1380
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Met Ile  Leu Glu Ile Cys Thr  Arg Tyr Arg Gly Asp  Gln Asp Ala
    1385                 1390                 1395

Thr Met  Ser Ile Leu Asp Ile  Ser Met Met Thr Gly  Phe Ala Pro
    1400                 1405                 1410

Asp Thr  Asp Asp Leu Lys Gln  Leu Ala Asn Gly Val  Asp Arg Tyr
    1415                 1420                 1425

Ile Ser  Lys Tyr Glu Leu Asp  Lys Ala Phe Ser Asp  Arg Asn Thr
    1430                 1435                 1440

Leu Ile  Ile Tyr Leu Asp Lys  Val Ser His Ser Glu  Asp Asp Cys
    1445                 1450                 1455

Leu Ala  Phe Lys Val His Gln  Tyr Phe Asn Val Glu  Leu Ile Gln
    1460                 1465                 1470

Pro Gly  Ala Val Lys Val Tyr  Ala Tyr Tyr Asn Leu  Glu Glu Ser
    1475                 1480                 1485

Cys Thr  Arg Phe Tyr His Pro  Glu Lys Glu Asp Gly  Lys Leu Asn
    1490                 1495                 1500

Lys Leu  Cys Arg Asp Glu Leu  Cys Arg Cys Ala Glu  Glu Asn Cys
    1505                 1510                 1515

Phe Ile  Gln Lys Ser Asp Asp  Lys Val Thr Leu Glu  Glu Arg Leu
    1520                 1525                 1530

Asp Lys  Ala Cys Glu Pro Gly  Val Asp Tyr Val Tyr  Lys Thr Arg
    1535                 1540                 1545

Leu Val  Lys Val Gln Leu Ser  Asn Asp Phe Asp Glu  Tyr Ile Met
    1550                 1555                 1560

Ala Ile  Glu Gln Thr Ile Lys  Ser Gly Ser Asp Glu  Val Gln Val
    1565                 1570                 1575

Gly Gln  Gln Arg Thr Phe Ile  Ser Pro Ile Lys Cys  Arg Glu Ala
    1580                 1585                 1590

Leu Lys  Leu Glu Glu Lys Lys  His Tyr Leu Met Trp  Gly Leu Ser
    1595                 1600                 1605

Ser Asp  Phe Trp Gly Glu Lys  Pro Asn Leu Ser Tyr  Ile Ile Gly
    1610                 1615                 1620

Lys Asp  Thr Trp Val Glu His  Trp Pro Glu Glu Asp  Glu Cys Gln
    1625                 1630                 1635

Asp Glu  Glu Asn Gln Lys Gln  Cys Gln Asp Leu Gly  Ala Phe Thr
    1640                 1645                 1650

Glu Ser  Met Val Val Phe Gly  Cys Pro Asn
    1655                 1660


<210> SEQ ID NO 7
<211> LENGTH: 203
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

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

Ser Glu Phe Pro Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu
            20                  25                  30

Pro Pro Lys Asn Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys
        35                  40                  45

Asp Ser Ile Thr Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys
    50                  55                  60
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```
Lys Gly Ile Cys Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp
65                  70                  75                  80

Phe Phe Ile Asp Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln
                85                  90                  95

Val Glu Ile Arg Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu
            100                 105                 110

Lys Val Arg Val Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala
        115                 120                 125

Thr Thr Lys Arg Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser
    130                 135                 140

Ser Leu Ser Val Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln
145                 150                 155                 160

Glu Val Glu Val Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly
                165                 170                 175

Val Arg Lys Ser Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys
            180                 185                 190

Thr Val Ala Val Arg Thr Leu Asp Pro Glu Arg
        195                 200


<210> SEQ ID NO 8
<211> LENGTH: 203
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

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

Ser Glu Phe Pro Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu
            20                  25                  30

Pro Pro Lys Asn Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys
        35                  40                  45

Asp Ser Ile Thr Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys
    50                  55                  60

Lys Gly Ile Cys Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp
65                  70                  75                  80

Phe Phe Ile Asp Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln
                85                  90                  95

Val Glu Ile Arg Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu
            100                 105                 110

Lys Val Arg Val Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala
        115                 120                 125

Thr Thr Lys Arg Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser
    130                 135                 140

Ser Leu Ser Val Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln
145                 150                 155                 160

Glu Val Glu Val Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly
                165                 170                 175

Val Arg Lys Ser Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys
            180                 185                 190

Thr Val Ala Val Arg Thr Leu Asp Pro Glu Arg
        195                 200
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<210> SEQ ID NO 9
<211> LENGTH: 1256
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Gly Pro Thr Ser Gly Pro Ser Leu Leu Leu Leu Leu Leu Thr His
1 5 10 15

Leu Pro Leu Ala Leu Gly Ser Pro Met Tyr Ser Ile Ile Thr Pro Asn
20 25 30

Ile Leu Arg Leu Glu Ser Glu Glu Thr Val Val Leu Glu Ala His Asp
35 40 45

Ala Gln Gly Asp Val Pro Val Thr Val Ile Val His Asp Phe Pro Gly
50 55 60

Lys Lys Leu Val Leu Ser Ser Glu Lys Thr Val Leu Thr Pro Ala Thr
65 70 75 80

Asn His Met Gly Asn Val Thr Phe Met Ile Pro Ala Asn Arg Glu Phe
85 90 95

Lys Ser Glu Lys Gly Arg Asn Lys Phe Val Thr Val Gln Ala Thr Phe
100 105 110

Gly Ala Gln Val Val Glu Lys Val Val Leu Val Ser Leu Gln Ser Gly
115 120 125

Tyr Leu Phe Ile Gln Thr Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr
130 135 140

Val Leu Tyr Arg Ile Phe Thr Val Asn His Lys Leu Leu Pro Val Gly
145 150 155 160

Arg Thr Val Met Val Asn Ile Glu Val Pro Ala Arg Gly Gly Pro Arg
165 170 175

Gly Ser Arg Gly Thr Gly Leu Gly Glu Ala Lys Arg Ser Arg Glu Thr
180 185 190

Glu Lys Asp Thr Pro Glu Gly Val Gln Phe Leu Tyr Gly Lys Lys Val
195 200 205

Glu Gly Thr Ala Phe Val Ile Phe Gly Ile Gln Asp Gly Glu Gln Arg
210 215 220

Ile Ser Leu Pro Glu Ser Leu Lys Arg Ile Pro Ile Glu Asp Gly Leu
225 230 235 240

Gly Glu Val Val Leu Ser Arg Lys Val Leu Leu Glu Gly Val His Asn
245 250 255

Pro Arg Ala Glu Asp Leu Val Gly Lys Ser Leu Tyr Val Ser Val Thr
260 265 270

Val Ile Leu His Ser Gly Ser Asp Met Val Gln Ala Glu Arg Ser Gly
275 280 285

Ile Pro Ile Val Thr Ser Pro Tyr Gln Ile His Phe Thr Lys Thr Pro
290 295 300

Lys Tyr Phe Lys Pro Gly Met Pro Phe Asp Leu Met Val Phe Val Thr
305 310 315 320

Asn Pro Asp Gly Ser Pro Ala Tyr Arg Val Pro Val Ala Val Gln Gly
325 330 335

Glu Asp Thr Val Gln Ser Leu Thr Gln Gly Asp Gly Val Ala Lys Leu
340 345 350

Ser Ile Asn Thr His Pro Ser Gln Lys Pro Leu Ser Ile Thr Val Arg
355 360 365

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Thr Lys Lys Gln Glu Leu Ser Glu Ala Glu Gln Ala Thr Ser Thr Met
    370                 375                 380

Gln Ala Leu Pro Tyr Ser Thr Val Gly Asn Ser Asn Asn Tyr Leu His
385                 390                 395                 400

Leu Ser Val Pro Arg Thr Glu Leu Arg Pro Gly Glu Thr Leu Asn Val
                405                 410                 415

Asn Phe Leu Leu Arg Met Asp Arg Ala His Glu Ala Lys Ile Arg Tyr
            420                 425                 430

Tyr Thr Tyr Leu Ile Met Asn Lys Gly Arg Leu Leu Lys Ala Gly Arg
        435                 440                 445

Gln Val Arg Glu Pro Gly Gln Asp Leu Val Val Leu Pro Leu Ser Ile
    450                 455                 460

Thr Thr Asp Phe Ile Pro Ser Phe Arg Leu Val Ala Tyr Tyr Thr Leu
465                 470                 475                 480

Ile Gly Ala Ser Gly Gln Arg Glu Val Val Ala Asp Ser Val Trp Val
                485                 490                 495

Asp Val Lys Asp Ser Cys Val Gly Ser Leu Ala Gly Gln Ser Gly Gln
            500                 505                 510

Ser Glu Asp Arg Gln Pro Val Pro Gly Gln Gln Met Thr Leu Lys Ile
        515                 520                 525

Glu Gly Asp His Gly Ala Arg Val Val Leu Val Ala Val Asp Lys Gly
    530                 535                 540

Val Phe Val Leu Asn Lys Lys Asn Lys Leu Thr Gln Ser Lys Ile Trp
545                 550                 555                 560

Asp Val Val Glu Lys Ala Asp Ile Gly Cys Thr Pro Gly Ser Gly Lys
                565                 570                 575

Asp Tyr Ala Gly Val Phe Ser Asp Ala Gly Leu Thr Phe Thr Ser Ser
            580                 585                 590

Ser Gly Gln Gln Thr Ala Gln Arg Ala Glu Leu Gln Cys Pro Gln Pro
        595                 600                 605

Ala Ala Arg Arg Arg Arg Ser Val Leu Leu Thr Glu Lys Arg Met Asp
    610                 615                 620

Lys Val Gly Lys Tyr Pro Lys Glu Leu Arg Lys Cys Cys Glu Asp Gly
625                 630                 635                 640

Met Arg Glu Asn Pro Met Arg Phe Ser Cys Gln Arg Arg Thr Arg Phe
                645                 650                 655

Ile Ser Leu Gly Glu Ala Cys Lys Lys Val Phe Leu Asp Cys Cys Asn
            660                 665                 670

Tyr Ile Thr Glu Leu Arg Arg Gln His Ala Arg Ala Gly His Leu Gly
        675                 680                 685

Leu Gly Arg Ser Asp Leu Asp Glu Asp Ile Ile Ala Glu Glu Asn Ile
    690                 695                 700

Val Ser Arg Ser Glu Phe Pro Glu Ser Trp Leu Trp Asn Val Glu Asp
705                 710                 715                 720

Leu Lys Glu Pro Pro Lys Asn Gly Ile Ser Thr Lys Leu Met Asn Ile
                725                 730                 735

Phe Leu Lys Asp Ser Ile Thr Thr Trp Glu Ile Leu Ala Val Ser Met
            740                 745                 750

Ser Asp Lys Lys Gly Glu Arg Gly Cys Trp Leu Val Pro Gly Arg Glu
        755                 760                 765

Ser Ala Ser His Ile Arg Gln Thr Arg Val Ser Gly Ser Gly Gly Arg
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    770                 775                 780

Gly Ser Gly Gly Ala Arg Gly Leu Val Ala Cys Cys Thr His Thr Cys
785                 790                 795                 800

Pro Asp Pro Phe Ser Pro Trp Gln Val Arg Val Glu Leu Leu His Asn
                805                 810                 815

Pro Ala Phe Cys Ser Leu Ala Thr Thr Lys Arg Arg His Gln Gln Thr
            820                 825                 830

Val Thr Ile Pro Pro Lys Ser Ser Leu Ser Val Pro Tyr Val Ile Val
        835                 840                 845

Pro Leu Lys Thr Gly Leu Gln Glu Val Glu Val Lys Ala Ala Val Tyr
    850                 855                 860

His His Phe Ile Ser Asp Gly Val Arg Lys Ser Leu Lys Val Val Pro
865                 870                 875                 880

Glu Gly Ile Arg Met Asn Lys Thr Val Ala Val Arg Thr Leu Asp Pro
                885                 890                 895

Glu Arg Leu Gly Arg Glu Gly Val Gln Lys Glu Asp Ile Pro Pro Ala
            900                 905                 910

Asp Leu Ser Asp Gln Val Pro Asp Thr Glu Ser Glu Thr Arg Ile Leu
        915                 920                 925

Leu Gln Gly Thr Pro Val Ala Gln Met Thr Glu Asp Ala Val Asp Ala
    930                 935                 940

Glu Arg Leu Lys His Leu Ile Val Thr Pro Ser Gly Cys Gly Glu Gln
945                 950                 955                 960

Asn Met Ile Gly Met Thr Pro Thr Val Ile Ala Val His Tyr Leu Asp
                965                 970                 975

Glu Thr Glu Gln Trp Glu Lys Phe Gly Leu Glu Lys Arg Gln Gly Ala
            980                 985                 990

Leu Glu Leu Ile Lys Lys Gly Tyr  Thr Gln Gln Leu Ala  Phe Arg Gln
        995                 1000                1005

Pro Ser  Ser Ala Phe Ala Ala  Phe Val Lys Arg Ala  Pro Ser Thr
    1010                1015                1020

Trp Leu  Thr Ala Tyr Val Val  Lys Val Phe Ser Leu  Ala Val Asn
    1025                1030                1035

Leu Ile  Ala Ile Asp Ser Gln  Val Leu Cys Gly Ala  Val Lys Trp
    1040                1045                1050

Leu Ile  Leu Glu Lys Gln Lys  Pro Asp Gly Val Phe  Gln Glu Asp
    1055                1060                1065

Ala Pro  Val Ile His Gln Glu  Met Ile Gly Gly Leu  Arg Asn Asn
    1070                1075                1080

Asn Glu  Lys Asp Met Ala Leu  Thr Ala Phe Val Leu  Ile Ser Leu
    1085                1090                1095

Gln Glu  Ala Lys Asp Ile Cys  Glu Glu Gln Val Asn  Ser Leu Pro
    1100                1105                1110

Gly Ser  Ile Thr Lys Ala Gly  Asp Phe Leu Glu Ala  Asn Tyr Met
    1115                1120                1125

Asn Leu  Gln Arg Ser Tyr Thr  Val Ala Ile Ala Gly  Tyr Ala Leu
    1130                1135                1140

Ala Gln  Met Gly Arg Leu Lys  Gly Pro Leu Leu Asn  Lys Phe Leu
    1145                1150                1155

Thr Thr  Ala Lys Asp Lys Asn  Arg Trp Glu Asp Pro  Gly Lys Gln
    1160                1165                1170
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Leu Tyr  Asn Val Glu Ala Thr  Ser Tyr Ala Leu Leu  Ala Leu Leu
    1175                 1180                 1185

Gln Leu  Lys Asp Phe Asp Phe  Val Pro Pro Val Val  Arg Trp Leu
    1190                 1195                 1200

Asn Glu  Gln Arg Tyr Tyr Gly  Gly Gly Tyr Gly Ser  Thr Gln Ala
    1205                 1210                 1215

Ser Gly  Pro Thr Ala Pro Arg  His Met His Pro Cys  Leu Leu Arg
    1220                 1225                 1230

Leu Pro  Thr Gly Leu Leu Glu  Lys Thr Leu Arg Pro  Ser Glu Ala
    1235                 1240                 1245

Val Leu  His Ser His Glu Pro  Val
    1250                 1255


<210> SEQ ID NO 10
<211> LENGTH: 349
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

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

Val Pro Asp Thr Glu Ser Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro
            20                  25                  30

Val Ala Gln Met Thr Glu Asp Ala Val Asp Ala Glu Arg Leu Lys His
        35                  40                  45

Leu Ile Val Thr Pro Ser Gly Cys Gly Glu Gln Asn Met Ile Gly Met
    50                  55                  60

Thr Pro Thr Val Ile Ala Val His Tyr Leu Asp Glu Thr Glu Gln Trp
65                  70                  75                  80

Glu Lys Phe Gly Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile Lys
                85                  90                  95

Lys Gly Tyr Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala Phe
            100                 105                 110

Ala Ala Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val
        115                 120                 125

Val Lys Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln
    130                 135                 140

Val Leu Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys Pro
145                 150                 155                 160

Asp Gly Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu Met Ile
                165                 170                 175

Gly Gly Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu Thr Ala Phe
            180                 185                 190

Val Leu Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys Glu Glu Gln Val
        195                 200                 205

Asn Ser Leu Pro Gly Ser Ile Thr Lys Ala Gly Asp Phe Leu Glu Ala
    210                 215                 220

Asn Tyr Met Asn Leu Gln Arg Ser Tyr Thr Val Ala Ile Ala Gly Tyr
225                 230                 235                 240

Ala Leu Ala Gln Met Gly Arg Leu Lys Gly Pro Leu Leu Asn Lys Phe
                245                 250                 255

Leu Thr Thr Ala Lys Asp Lys Asn Arg Trp Glu Asp Pro Gly Lys Gln
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            260                 265                 270

Leu Tyr Asn Val Glu Ala Thr Ser Tyr Ala Leu Leu Ala Leu Leu Gln
        275                 280                 285

Leu Lys Asp Phe Asp Phe Val Pro Pro Val Val Arg Trp Leu Asn Glu
    290                 295                 300

Gln Arg Tyr Tyr Gly Gly Gly Tyr Gly Ser Thr Gln Ala Thr Phe Met
305                 310                 315                 320

Val Phe Gln Ala Leu Ala Gln Tyr Gln Lys Asp Ala Pro Asp His Gln
                325                 330                 335

Glu Leu Asn Leu Asp Val Ser Leu Gln Leu Pro Ser Arg
            340                 345


<210> SEQ ID NO 11
<211> LENGTH: 355
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

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

Val Pro Asp Thr Glu Ser Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro
            20                  25                  30

Val Ala Gln Met Thr Glu Asp Ala Val Asp Ala Glu Arg Leu Lys His
        35                  40                  45

Leu Ile Val Thr Pro Ser Gly Cys Gly Glu Gln Asn Met Ile Gly Met
    50                  55                  60

Thr Pro Thr Val Ile Ala Val His Tyr Leu Asp Glu Thr Glu Gln Trp
65                  70                  75                  80

Glu Lys Phe Gly Leu Glu Lys Arg Gln Gly Ala Leu Glu Leu Ile Lys
                85                  90                  95

Lys Gly Tyr Thr Gln Gln Leu Ala Phe Arg Gln Pro Ser Ser Ala Phe
            100                 105                 110

Ala Ala Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val
        115                 120                 125

Val Lys Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln
    130                 135                 140

Val Leu Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys Pro
145                 150                 155                 160

Asp Gly Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu Met Ile
                165                 170                 175

Gly Gly Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu Thr Ala Phe
            180                 185                 190

Val Leu Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys Glu Glu Gln Val
        195                 200                 205

Asn Ser Leu Pro Gly Ser Ile Thr Lys Ala Gly Asp Phe Leu Glu Ala
    210                 215                 220

Asn Tyr Met Asn Leu Gln Arg Ser Tyr Thr Val Ala Ile Ala Gly Tyr
225                 230                 235                 240

Ala Leu Ala Gln Met Gly Arg Leu Lys Gly Pro Leu Leu Asn Lys Phe
                245                 250                 255

Leu Thr Thr Ala Lys Asp Lys Asn Arg Trp Glu Asp Pro Gly Lys Gln
            260                 265                 270
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Leu Tyr Asn Val Glu Ala Thr Ser Tyr Ala Leu Leu Ala Leu Leu Gln
        275                 280                 285

Leu Lys Asp Phe Asp Phe Val Pro Pro Val Val Arg Trp Leu Asn Glu
    290                 295                 300

Gln Arg Tyr Tyr Gly Gly Gly Tyr Gly Ser Thr Gln Ala Ser Gly Pro
305                 310                 315                 320

Thr Ala Pro Arg His Met His Pro Cys Leu Leu Arg Leu Pro Thr Gly
                325                 330                 335

Leu Leu Glu Lys Thr Leu Arg Pro Ser Glu Ala Val Leu His Ser His
            340                 345                 350

Glu Pro Val
        355


<210> SEQ ID NO 12
<211> LENGTH: 505
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Lys Ile Val Leu Asp Pro Ser Gly Ser Met Asn Ile Tyr Leu Val Leu
1               5                   10                  15

Asp Gly Ser Asp Ser Ile Gly Ala Ser Asn Phe Thr Gly Ala Lys Lys
            20                  25                  30

Cys Leu Val Asn Leu Ile Glu Lys Val Ala Ser Tyr Gly Val Lys Pro
        35                  40                  45

Arg Tyr Gly Leu Val Thr Tyr Ala Thr Tyr Pro Lys Ile Trp Val Lys
    50                  55                  60

Val Ser Glu Ala Asp Ser Ser Asn Ala Asp Trp Val Thr Lys Gln Leu
65                  70                  75                  80

Asn Glu Ile Asn Tyr Glu Asp His Lys Leu Lys Ser Gly Thr Asn Thr
                85                  90                  95

Lys Lys Ala Leu Gln Ala Val Tyr Ser Met Met Ser Trp Pro Asp Asp
            100                 105                 110

Val Pro Pro Glu Gly Trp Asn Arg Thr Arg His Val Ile Ile Leu Met
        115                 120                 125

Thr Asp Gly Leu His Asn Met Gly Gly Asp Pro Ile Thr Val Ile Asp
    130                 135                 140

Glu Ile Arg Asp Leu Leu Tyr Ile Gly Lys Asp Arg Lys Asn Pro Arg
145                 150                 155                 160

Glu Asp Tyr Leu Asp Val Tyr Val Phe Gly Val Gly Pro Leu Val Asn
                165                 170                 175

Gln Val Asn Ile Asn Ala Leu Ala Ser Lys Lys Asp Asn Glu Gln His
            180                 185                 190

Val Phe Lys Val Lys Asp Met Glu Asn Leu Glu Asp Val Phe Tyr Gln
        195                 200                 205

Met Ile Asp Glu Ser Gln Ser Leu Ser Leu Cys Gly Met Val Trp Glu
    210                 215                 220

His Arg Lys Gly Thr Asp Tyr His Lys Gln Pro Trp Gln Ala Lys Ile
225                 230                 235                 240

Ser Val Ile Arg Pro Ser Lys Gly His Glu Ser Cys Met Gly Ala Val
                245                 250                 255

Val Ser Glu Tyr Phe Val Leu Thr Ala Ala His Cys Phe Thr Val Asp
            260                 265                 270
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Asp Lys Glu His Ser Ile Lys Val Ser Val Gly Gly Glu Lys Arg Asp
        275                 280                 285

Leu Glu Ile Glu Val Val Leu Phe His Pro Asn Tyr Asn Ile Asn Gly
    290                 295                 300

Lys Lys Glu Ala Gly Ile Pro Glu Phe Tyr Asp Tyr Asp Val Ala Leu
305                 310                 315                 320

Ile Lys Leu Lys Asn Lys Leu Lys Tyr Gly Gln Thr Ile Arg Pro Ile
                325                 330                 335

Cys Leu Pro Cys Thr Glu Gly Thr Thr Arg Ala Leu Arg Leu Pro Pro
            340                 345                 350

Thr Thr Thr Cys Gln Gln Gln Lys Glu Glu Leu Leu Pro Ala Gln Asp
        355                 360                 365

Ile Lys Ala Leu Phe Val Ser Glu Glu Glu Lys Lys Leu Thr Arg Lys
    370                 375                 380

Glu Val Tyr Ile Lys Asn Gly Asp Lys Lys Gly Ser Cys Glu Arg Asp
385                 390                 395                 400

Ala Gln Tyr Ala Pro Gly Tyr Asp Lys Val Lys Asp Ile Ser Glu Val
                405                 410                 415

Val Thr Pro Arg Phe Leu Cys Thr Gly Gly Val Ser Pro Tyr Ala Asp
            420                 425                 430

Pro Asn Thr Cys Arg Gly Asp Ser Gly Gly Pro Leu Ile Val His Lys
        435                 440                 445

Arg Ser Arg Phe Ile Gln Val Gly Val Ile Ser Trp Gly Val Val Asp
    450                 455                 460

Val Cys Lys Asn Gln Lys Arg Gln Lys Gln Val Pro Ala His Ala Arg
465                 470                 475                 480

Asp Phe His Ile Asn Leu Phe Gln Val Leu Pro Trp Leu Lys Glu Lys
                485                 490                 495

Leu Gln Asp Glu Asp Leu Gly Phe Leu
            500                 505


<210> SEQ ID NO 13
<211> LENGTH: 1231
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Met Arg Leu Leu Ala Lys Ile Ile Cys Leu Met Leu Trp Ala Ile Cys
1               5                   10                  15

Val Ala Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile
            20                  25                  30

Leu Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala
        35                  40                  45

Ile Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met
    50                  55                  60

Val Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys
65                  70                  75                  80

Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
                85                  90                  95

Thr Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr
            100                 105                 110

Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu
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        115                 120                 125

Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
    130                 135                 140

Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser
145                 150                 155                 160

Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe
                165                 170                 175

Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys
            180                 185                 190

Ser Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile
        195                 200                 205

Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys
    210                 215                 220

Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly
225                 230                 235                 240

Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp
                245                 250                 255

Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile
            260                 265                 270

Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp
        275                 280                 285

Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly
    290                 295                 300

Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys
305                 310                 315                 320

Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu Tyr
                325                 330                 335

His Glu Asn Met Arg Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr
            340                 345                 350

Tyr Ser Tyr Tyr Cys Asp Glu His Phe Glu Thr Pro Ser Gly Ser Tyr
        355                 360                 365

Trp Asp His Ile His Cys Thr Gln Asp Gly Trp Ser Pro Ala Val Pro
    370                 375                 380

Cys Leu Arg Lys Cys Tyr Phe Pro Tyr Leu Glu Asn Gly Tyr Asn Gln
385                 390                 395                 400

Asn His Gly Arg Lys Phe Val Gln Gly Lys Ser Ile Asp Val Ala Cys
                405                 410                 415

His Pro Gly Tyr Ala Leu Pro Lys Ala Gln Thr Thr Val Thr Cys Met
            420                 425                 430

Glu Asn Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Lys Thr Cys
        435                 440                 445

Ser Lys Ser Ser Ile Asp Ile Glu Asn Gly Phe Ile Ser Glu Ser Gln
    450                 455                 460

Tyr Thr Tyr Ala Leu Lys Glu Lys Ala Lys Tyr Gln Cys Lys Leu Gly
465                 470                 475                 480

Tyr Val Thr Ala Asp Gly Glu Thr Ser Gly Ser Ile Thr Cys Gly Lys
                485                 490                 495

Asp Gly Trp Ser Ala Gln Pro Thr Cys Ile Lys Ser Cys Asp Ile Pro
            500                 505                 510

Val Phe Met Asn Ala Arg Thr Lys Asn Asp Phe Thr Trp Phe Lys Leu
        515                 520                 525
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Asn Asp Thr Leu Asp Tyr Glu Cys His Asp Gly Tyr Glu Ser Asn Thr
    530                 535                 540

Gly Ser Thr Thr Gly Ser Ile Val Cys Gly Tyr Asn Gly Trp Ser Asp
545                 550                 555                 560

Leu Pro Ile Cys Tyr Glu Arg Glu Cys Glu Leu Pro Lys Ile Asp Val
                565                 570                 575

His Leu Val Pro Asp Arg Lys Lys Asp Gln Tyr Lys Val Gly Glu Val
            580                 585                 590

Leu Lys Phe Ser Cys Lys Pro Gly Phe Thr Ile Val Gly Pro Asn Ser
        595                 600                 605

Val Gln Cys Tyr His Phe Gly Leu Ser Pro Asp Leu Pro Ile Cys Lys
    610                 615                 620

Glu Gln Val Gln Ser Cys Gly Pro Pro Pro Glu Leu Leu Asn Gly Asn
625                 630                 635                 640

Val Lys Glu Lys Thr Lys Glu Glu Tyr Gly His Ser Glu Val Val Glu
                645                 650                 655

Tyr Tyr Cys Asn Pro Arg Phe Leu Met Lys Gly Pro Asn Lys Ile Gln
            660                 665                 670

Cys Val Asp Gly Glu Trp Thr Thr Leu Pro Val Cys Ile Val Glu Glu
        675                 680                 685

Ser Thr Cys Gly Asp Ile Pro Glu Leu Glu His Gly Trp Ala Gln Leu
    690                 695                 700

Ser Ser Pro Pro Tyr Tyr Tyr Gly Asp Ser Val Glu Phe Asn Cys Ser
705                 710                 715                 720

Glu Ser Phe Thr Met Ile Gly His Arg Ser Ile Thr Cys Ile His Gly
                725                 730                 735

Val Trp Thr Gln Leu Pro Gln Cys Val Ala Ile Asp Lys Leu Lys Lys
            740                 745                 750

Cys Lys Ser Ser Asn Leu Ile Ile Leu Glu Glu His Leu Lys Asn Lys
        755                 760                 765

Lys Glu Phe Asp His Asn Ser Asn Ile Arg Tyr Arg Cys Arg Gly Lys
    770                 775                 780

Glu Gly Trp Ile His Thr Val Cys Ile Asn Gly Arg Trp Asp Pro Glu
785                 790                 795                 800

Val Asn Cys Ser Met Ala Gln Ile Gln Leu Cys Pro Pro Pro Pro Gln
                805                 810                 815

Ile Pro Asn Ser His Asn Met Thr Thr Thr Leu Asn Tyr Arg Asp Gly
            820                 825                 830

Glu Lys Val Ser Val Leu Cys Gln Glu Asn Tyr Leu Ile Gln Glu Gly
        835                 840                 845

Glu Glu Ile Thr Cys Lys Asp Gly Arg Trp Gln Ser Ile Pro Leu Cys
    850                 855                 860

Val Glu Lys Ile Pro Cys Ser Gln Pro Pro Gln Ile Glu His Gly Thr
865                 870                 875                 880

Ile Asn Ser Ser Arg Ser Ser Gln Glu Ser Tyr Ala His Gly Thr Lys
                885                 890                 895

Leu Ser Tyr Thr Cys Glu Gly Gly Phe Arg Ile Ser Glu Glu Asn Glu
            900                 905                 910

Thr Thr Cys Tyr Met Gly Lys Trp Ser Ser Pro Pro Gln Cys Glu Gly
        915                 920                 925
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Leu Pro Cys Lys Ser Pro Pro Glu Ile Ser His Gly Val Val Ala His
    930                 935                 940

Met Ser Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr Lys Cys Phe
945                 950                 955                 960

Glu Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu
                965                 970                 975

Lys Trp Ser His Pro Pro Ser Cys Ile Lys Thr Asp Cys Leu Ser Leu
            980                 985                 990

Pro Ser Phe Glu Asn Ala Ile Pro  Met Gly Glu Lys Lys  Asp Val Tyr
        995                 1000                1005

Lys Ala  Gly Glu Gln Val Thr  Tyr Thr Cys Ala Thr  Tyr Tyr Lys
    1010                1015                1020

Met Asp  Gly Ala Ser Asn Val  Thr Cys Ile Asn Ser  Arg Trp Thr
    1025                1030                1035

Gly Arg  Pro Thr Cys Arg Asp  Thr Ser Cys Val Asn  Pro Pro Thr
    1040                1045                1050

Val Gln  Asn Ala Tyr Ile Val  Ser Arg Gln Met Ser  Lys Tyr Pro
    1055                1060                1065

Ser Gly  Glu Arg Val Arg Tyr  Gln Cys Arg Ser Pro  Tyr Glu Met
    1070                1075                1080

Phe Gly  Asp Glu Glu Val Met  Cys Leu Asn Gly Asn  Trp Thr Glu
    1085                1090                1095

Pro Pro  Gln Cys Lys Asp Ser  Thr Gly Lys Cys Gly  Pro Pro Pro
    1100                1105                1110

Pro Ile  Asp Asn Gly Asp Ile  Thr Ser Phe Pro Leu  Ser Val Tyr
    1115                1120                1125

Ala Pro  Ala Ser Ser Val Glu  Tyr Gln Cys Gln Asn  Leu Tyr Gln
    1130                1135                1140

Leu Glu  Gly Asn Lys Arg Ile  Thr Cys Arg Asn Gly  Gln Trp Ser
    1145                1150                1155

Glu Pro  Pro Lys Cys Leu His  Pro Cys Val Ile Ser  Arg Glu Ile
    1160                1165                1170

Met Glu  Asn Tyr Asn Ile Ala  Leu Arg Trp Thr Ala  Lys Gln Lys
    1175                1180                1185

Leu Tyr  Ser Arg Thr Gly Glu  Ser Val Glu Phe Val  Cys Lys Arg
    1190                1195                1200

Gly Tyr  Arg Leu Ser Ser Arg  Ser His Thr Leu Arg  Thr Thr Cys
    1205                1210                1215

Trp Asp  Gly Lys Leu Glu Tyr  Pro Thr Cys Ala Lys  Arg
    1220                1225                1230


<210> SEQ ID NO 14
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Arg Leu Leu Ala Lys Ile Ile Cys Leu Met Leu Trp Ala Ile Cys
1               5                   10                  15

Val Ala Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile
            20                  25                  30

Leu Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala
        35                  40                  45
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Ile Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met
    50                  55                  60

Val Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys
65                  70                  75                  80

Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
                85                  90                  95

Thr Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr
            100                 105                 110

Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu
        115                 120                 125

Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
    130                 135                 140

Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser
145                 150                 155                 160

Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe
                165                 170                 175

Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys
            180                 185                 190

Ser Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile
        195                 200                 205

Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys
    210                 215                 220

Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly
225                 230                 235                 240

Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp
                245                 250                 255

Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile
            260                 265                 270

Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp
        275                 280                 285

Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly
    290                 295                 300

Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys
305                 310                 315                 320

Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu Tyr
                325                 330                 335

His Glu Asn Met Arg Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr
            340                 345                 350

Tyr Ser Tyr Tyr Cys Asp Glu His Phe Glu Thr Pro Ser Gly Ser Tyr
        355                 360                 365

Trp Asp His Ile His Cys Thr Gln Asp Gly Trp Ser Pro Ala Val Pro
    370                 375                 380

Cys Leu Arg Lys Cys Tyr Phe Pro Tyr Leu Glu Asn Gly Tyr Asn Gln
385                 390                 395                 400

Asn His Gly Arg Lys Phe Val Gln Gly Lys Ser Ile Asp Val Ala Cys
                405                 410                 415

His Pro Gly Tyr Ala Leu Pro Lys Ala Gln Thr Thr Val Thr Cys Met
            420                 425                 430

Glu Asn Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Ser Phe Thr
        435                 440                 445
```

-continued

Leu

<210> SEQ ID NO 15
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Arg Leu Leu Ala Lys Ile Ile Cys Leu Met Leu Trp Ala Ile Cys
1 5 10 15

Val Ala Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile
20 25 30

Leu Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala
35 40 45

Ile Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met
50 55 60

Val Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys
65 70 75 80

Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
85 90 95

Thr Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr
100 105 110

Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu
115 120 125

Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
130 135 140

Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser
145 150 155 160

Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe
165 170 175

Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys
180 185 190

Ser Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile
195 200 205

Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys
210 215 220

Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly
225 230 235 240

Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp
245 250 255

Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile
260 265 270

Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp
275 280 285

Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly
290 295 300

Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys
305 310 315 320

Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu Tyr
325 330 335

His Glu Asn Met Arg Arg
340

-continued

<210> SEQ ID NO 16
<211> LENGTH: 930
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met Lys Pro Pro Arg Pro Val Arg Thr Cys Ser Lys Val Leu Val Leu
1 5 10 15

Leu Ser Leu Leu Ala Ile His Gln Thr Thr Thr Ala Glu Lys Asn Gly
20 25 30

Ile Asp Ile Tyr Ser Leu Thr Val Asp Ser Arg Val Ser Ser Arg Phe
35 40 45

Ala His Thr Val Val Thr Ser Arg Val Val Asn Arg Ala Asn Thr Val
50 55 60

Gln Glu Ala Thr Phe Gln Met Glu Leu Pro Lys Lys Ala Phe Ile Thr
65 70 75 80

Asn Phe Ser Met Asn Ile Asp Gly Met Thr Tyr Pro Gly Ile Ile Lys
85 90 95

Glu Lys Ala Glu Ala Gln Ala Gln Tyr Ser Ala Ala Val Ala Lys Gly
100 105 110

Lys Asn Ala Gly Leu Val Lys Ala Thr Gly Arg Asn Met Glu Gln Phe
115 120 125

Gln Val Ser Val Ser Val Ala Pro Asn Ala Lys Ile Thr Phe Glu Leu
130 135 140

Val Tyr Glu Glu Leu Leu Lys Arg Arg Leu Gly Val Tyr Glu Leu Leu
145 150 155 160

Leu Lys Val Arg Pro Gln Gln Leu Val Lys His Leu Gln Met Asp Ile
165 170 175

His Ile Phe Glu Pro Gln Gly Ile Ser Phe Leu Glu Thr Glu Ser Thr
180 185 190

Phe Met Thr Asn Gln Leu Val Asp Ala Leu Thr Thr Trp Gln Asn Lys
195 200 205

Thr Lys Ala His Ile Arg Phe Lys Pro Thr Leu Ser Gln Gln Gln Lys
210 215 220

Ser Pro Glu Gln Gln Glu Thr Val Leu Asp Gly Asn Leu Ile Ile Arg
225 230 235 240

Tyr Asp Val Asp Arg Ala Ile Ser Gly Gly Ser Ile Gln Ile Glu Asn
245 250 255

Gly Tyr Phe Val His Tyr Phe Ala Pro Glu Gly Leu Thr Thr Met Pro
260 265 270

Lys Asn Val Val Phe Val Ile Asp Lys Ser Gly Ser Met Ser Gly Arg
275 280 285

Lys Ile Gln Gln Thr Arg Glu Ala Leu Ile Lys Ile Leu Asp Asp Leu
290 295 300

Ser Pro Arg Asp Gln Phe Asn Leu Ile Val Phe Ser Thr Glu Ala Thr
305 310 315 320

Gln Trp Arg Pro Ser Leu Val Pro Ala Ser Ala Glu Asn Val Asn Lys
325 330 335

Ala Arg Ser Phe Ala Ala Gly Ile Gln Ala Leu Gly Gly Thr Asn Ile
340 345 350

Asn Asp Ala Met Leu Met Ala Val Gln Leu Leu Asp Ser Ser Asn Gln
355 360 365

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```
Glu Glu Arg Leu Pro Glu Gly Ser Val Ser Leu Ile Ile Leu Leu Thr
    370                 375                 380

Asp Gly Asp Pro Thr Val Gly Glu Thr Asn Pro Arg Ser Ile Gln Asn
385                 390                 395                 400

Asn Val Arg Glu Ala Val Ser Gly Arg Tyr Ser Leu Phe Cys Leu Gly
                405                 410                 415

Phe Gly Phe Asp Val Ser Tyr Ala Phe Leu Glu Lys Leu Ala Leu Asp
            420                 425                 430

Asn Gly Gly Leu Ala Arg Arg Ile His Glu Asp Ser Asp Ser Ala Leu
        435                 440                 445

Gln Leu Gln Asp Phe Tyr Gln Glu Val Ala Asn Pro Leu Leu Thr Ala
    450                 455                 460

Val Thr Phe Glu Tyr Pro Ser Asn Ala Val Glu Glu Val Thr Gln Asn
465                 470                 475                 480

Asn Phe Arg Leu Leu Phe Lys Gly Ser Glu Met Val Val Ala Gly Lys
                485                 490                 495

Leu Gln Asp Arg Gly Pro Asp Val Leu Thr Ala Thr Val Ser Gly Lys
            500                 505                 510

Leu Pro Thr Gln Asn Ile Thr Phe Gln Thr Glu Ser Ser Val Ala Glu
        515                 520                 525

Gln Glu Ala Glu Phe Gln Ser Pro Lys Tyr Ile Phe His Asn Phe Met
    530                 535                 540

Glu Arg Leu Trp Ala Tyr Leu Thr Ile Gln Gln Leu Leu Glu Gln Thr
545                 550                 555                 560

Val Ser Ala Ser Asp Ala Asp Gln Gln Ala Leu Arg Asn Gln Ala Leu
                565                 570                 575

Asn Leu Ser Leu Ala Tyr Ser Phe Val Thr Pro Leu Thr Ser Met Val
            580                 585                 590

Val Thr Lys Pro Asp Asp Gln Glu Gln Ser Gln Val Ala Glu Lys Pro
        595                 600                 605

Met Glu Gly Glu Ser Arg Asn Arg Asn Val His Ser Gly Ser Thr Phe
    610                 615                 620

Phe Lys Tyr Tyr Leu Gln Gly Ala Lys Ile Pro Lys Pro Glu Ala Ser
625                 630                 635                 640

Phe Ser Pro Arg Arg Gly Trp Asn Arg Gln Ala Gly Ala Ala Gly Ser
                645                 650                 655

Arg Met Asn Phe Arg Pro Gly Val Leu Ser Ser Arg Gln Leu Gly Leu
            660                 665                 670

Pro Gly Pro Pro Asp Val Pro Asp His Ala Ala Tyr His Pro Phe Arg
        675                 680                 685

Arg Leu Ala Ile Leu Pro Ala Ser Ala Pro Pro Ala Thr Ser Asn Pro
    690                 695                 700

Asp Pro Ala Val Ser Arg Val Met Asn Met Lys Ile Glu Glu Thr Thr
705                 710                 715                 720

Met Thr Thr Gln Thr Pro Ala Pro Ile Gln Ala Pro Ser Ala Ile Leu
                725                 730                 735

Pro Leu Pro Gly Gln Ser Val Glu Arg Leu Cys Val Asp Pro Arg His
            740                 745                 750

Arg Gln Gly Pro Val Asn Leu Leu Ser Asp Pro Glu Gln Gly Val Glu
        755                 760                 765
```

-continued

```
Val Thr Gly Gln Tyr Glu Arg Glu Lys Ala Gly Phe Ser Trp Ile Glu
    770                 775                 780

Val Thr Phe Lys Asn Pro Leu Val Trp Val His Ala Ser Pro Glu His
785                 790                 795                 800

Val Val Val Thr Arg Asn Arg Arg Ser Ser Ala Tyr Lys Trp Lys Glu
                805                 810                 815

Thr Leu Phe Ser Val Met Pro Gly Leu Lys Met Thr Met Asp Lys Thr
            820                 825                 830

Gly Leu Leu Leu Leu Ser Asp Pro Asp Lys Val Thr Ile Gly Leu Leu
        835                 840                 845

Phe Trp Asp Gly Arg Gly Glu Gly Leu Arg Leu Leu Leu Arg Asp Thr
    850                 855                 860

Asp Arg Phe Ser Ser His Val Gly Gly Thr Leu Gly Gln Phe Tyr Gln
865                 870                 875                 880

Glu Val Leu Trp Gly Ser Pro Ala Ala Ser Asp Asp Gly Arg Arg Thr
                885                 890                 895

Leu Arg Val Gln Gly Asn Asp His Ser Ala Thr Arg Glu Arg Arg Leu
            900                 905                 910

Asp Tyr Gln Glu Gly Pro Pro Gly Val Glu Ile Ser Cys Trp Ser Val
        915                 920                 925

Glu Leu
    930


<210> SEQ ID NO 17
<211> LENGTH: 242
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

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

Asp Pro Ala Val Ser Arg Val Met Asn Met Lys Ile Glu Glu Thr Thr
            20                  25                  30

Met Thr Thr Gln Thr Pro Ala Pro Ile Gln Ala Pro Ser Ala Ile Leu
        35                  40                  45

Pro Leu Pro Gly Gln Ser Val Glu Arg Leu Cys Val Asp Pro Arg His
    50                  55                  60

Arg Gln Gly Pro Val Asn Leu Leu Ser Asp Pro Glu Gln Gly Val Glu
65                  70                  75                  80

Val Thr Gly Gln Tyr Glu Arg Glu Lys Ala Gly Phe Ser Trp Ile Glu
                85                  90                  95

Val Thr Phe Lys Asn Pro Leu Val Trp Val His Ala Ser Pro Glu His
            100                 105                 110

Val Val Val Thr Arg Asn Arg Arg Ser Ser Ala Tyr Lys Trp Lys Glu
        115                 120                 125

Thr Leu Phe Ser Val Met Pro Gly Leu Lys Met Thr Met Asp Lys Thr
    130                 135                 140

Gly Leu Leu Leu Leu Ser Asp Pro Asp Lys Val Thr Ile Gly Leu Leu
145                 150                 155                 160

Phe Trp Asp Gly Arg Gly Glu Gly Leu Arg Leu Leu Leu Arg Asp Thr
                165                 170                 175

Asp Arg Phe Ser Ser His Val Gly Gly Thr Leu Gly Gln Phe Tyr Gln
            180                 185                 190
```

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```
Glu Val Leu Trp Gly Ser Pro Ala Ala Ser Asp Asp Gly Arg Arg Thr
        195                 200                 205

Leu Arg Val Gln Gly Asn Asp His Ser Ala Thr Arg Glu Arg Arg Leu
    210                 215                 220

Asp Tyr Gln Glu Gly Pro Pro Gly Val Glu Ile Ser Cys Trp Ser Val
225                 230                 235                 240

Glu Leu
```

<210> SEQ ID NO 18
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

```
Arg Leu Ala Ile Leu Pro Ala Ser Ala Pro Pro Ala Thr Ser Asn Pro
1               5                   10                  15

Asp Pro Ala Val Ser Arg Val Met Asn Met Lys Ile Glu Glu Thr Thr
            20                  25                  30

Met Thr Thr Gln Thr Pro Ala Cys Pro Ser Cys Ser Arg Ser Arg Ala
        35                  40                  45

Pro Ala Val Pro Ala Pro Ile Gln Ala Pro Ser Ala Ile Leu Pro Leu
    50                  55                  60

Pro Gly Gln Ser Val Glu Arg Leu Cys Val Asp Pro Arg His Arg Gln
65                  70                  75                  80

Gly Pro Val Asn Leu Leu Ser Asp Pro Glu Gln Gly Val Glu Val Thr
                85                  90                  95

Gly Gln Tyr Glu Arg Glu Lys Ala Gly Phe Ser Trp Ile Glu Val Thr
            100                 105                 110

Phe Lys Asn Pro Leu Val Trp Val His Ala Ser Pro Glu His Val Val
        115                 120                 125

Val Thr Arg Asn Arg Arg Ser Ser Ala Tyr Lys Trp Lys Glu Thr Leu
    130                 135                 140

Phe Ser Val Met Pro Gly Leu Lys Met Thr Met Asp Lys Thr Gly Leu
145                 150                 155                 160

Leu Leu Leu Ser Asp Pro Asp Lys Val Thr Ile Gly Leu Leu Phe Trp
                165                 170                 175

Asp Gly Arg Gly Glu Gly Leu Arg Leu Leu Leu Arg Asp Thr Asp Arg
            180                 185                 190

Phe Ser Ser His Val Gly Gly Thr Leu Gly Gln Phe Tyr Gln Glu Val
        195                 200                 205

Leu Trp Gly Ser Pro Ala Ala Ser Asp Asp Gly Arg Arg Thr Leu Arg
    210                 215                 220

Val Gln Gly Asn Asp His Ser Ala Thr Arg Glu Arg Arg Leu Asp Tyr
225                 230                 235                 240

Gln Glu Gly Pro Pro Gly Val Glu Ile Ser Cys Trp Ser Val Glu Leu
                245                 250                 255
```

<210> SEQ ID NO 19
<211> LENGTH: 281
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

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

His Met Ala Met Trp Ser Thr Arg Phe Ala Thr Ser Val Arg Thr Asn
            20                  25                  30

Ala Val Gln Arg Ile Leu Gly Gly His Leu Asp Ala Lys Gly Ser Phe
        35                  40                  45

Pro Trp Gln Ala Lys Met Val Ser His His Asn Leu Thr Thr Gly Ala
    50                  55                  60

Thr Leu Ile Asn Glu Gln Trp Leu Leu Thr Thr Ala Lys Asn Leu Phe
65                  70                  75                  80

Leu Asn His Ser Glu Asn Ala Thr Ala Lys Asp Ile Ala Pro Thr Leu
                85                  90                  95

Thr Leu Tyr Val Gly Lys Lys Gln Leu Val Glu Ile Glu Lys Val Val
            100                 105                 110

Leu His Pro Asn Tyr Ser Gln Val Asp Ile Gly Leu Ile Lys Leu Lys
        115                 120                 125

Gln Lys Val Ser Val Asn Glu Arg Val Met Pro Ile Cys Leu Pro Ser
    130                 135                 140

Lys Asp Tyr Ala Glu Val Gly Arg Val Gly Tyr Val Ser Gly Trp Gly
145                 150                 155                 160

Arg Asn Ala Asn Phe Lys Phe Thr Asp His Leu Lys Tyr Val Met Leu
                165                 170                 175

Pro Val Ala Asp Gln Asp Gln Cys Ile Arg His Tyr Glu Gly Ser Thr
            180                 185                 190

Val Pro Glu Lys Lys Thr Pro Lys Ser Pro Val Gly Val Gln Pro Ile
        195                 200                 205

Leu Asn Glu His Thr Phe Cys Ala Gly Met Ser Lys Tyr Gln Glu Asp
    210                 215                 220

Thr Cys Tyr Gly Asp Ala Gly Ser Ala Phe Ala Val His Asp Leu Glu
225                 230                 235                 240

Glu Asp Thr Trp Tyr Ala Thr Gly Ile Leu Ser Phe Asp Lys Ser Cys
                245                 250                 255

Ala Val Ala Glu Tyr Gly Val Tyr Val Lys Val Thr Ser Ile Gln Asp
            260                 265                 270

Trp Val Gln Lys Thr Ile Ala Glu Asn
        275                 280


<210> SEQ ID NO 20
<211> LENGTH: 583
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Lys Leu Leu His Val Phe Leu Leu Phe Leu Cys Phe His Leu Arg
1               5                  10                  15

Phe Cys Lys Val Thr Tyr Thr Ser Gln Glu Asp Leu Val Glu Lys Lys
            20                  25                  30

Cys Leu Ala Lys Lys Tyr Thr His Leu Ser Cys Asp Lys Val Phe Cys
        35                  40                  45

Gln Pro Trp Gln Arg Cys Ile Glu Gly Thr Cys Val Cys Lys Leu Pro
    50                  55                  60

Tyr Gln Cys Pro Lys Asn Gly Thr Ala Val Cys Ala Thr Asn Arg Arg
65                  70                  75                  80
```

-continued

```
Ser Phe Pro Thr Tyr Cys Gln Gln Lys Ser Leu Glu Cys Leu His Pro
                85                  90                  95

Gly Thr Lys Phe Leu Asn Asn Gly Thr Cys Thr Ala Glu Gly Lys Phe
            100                 105                 110

Ser Val Ser Leu Lys His Gly Asn Thr Asp Ser Glu Gly Ile Val Glu
        115                 120                 125

Val Lys Leu Val Asp Gln Asp Lys Thr Met Phe Ile Cys Lys Ser Ser
    130                 135                 140

Trp Ser Met Arg Glu Ala Asn Val Ala Cys Leu Asp Leu Gly Phe Gln
145                 150                 155                 160

Gln Gly Ala Asp Thr Gln Arg Arg Phe Lys Leu Ser Asp Leu Ser Ile
                165                 170                 175

Asn Ser Thr Glu Cys Leu His Val His Cys Arg Gly Leu Glu Thr Ser
            180                 185                 190

Leu Ala Glu Cys Thr Phe Thr Lys Arg Arg Thr Met Gly Tyr Gln Asp
        195                 200                 205

Phe Ala Asp Val Val Cys Tyr Thr Gln Lys Ala Asp Ser Pro Met Asp
    210                 215                 220

Asp Phe Phe Gln Cys Val Asn Gly Lys Tyr Ile Ser Gln Met Lys Ala
225                 230                 235                 240

Cys Asp Gly Ile Asn Asp Cys Gly Asp Gln Ser Asp Glu Leu Cys Cys
                245                 250                 255

Lys Ala Cys Gln Gly Lys Gly Phe His Cys Lys Ser Gly Val Cys Ile
            260                 265                 270

Pro Ser Gln Tyr Gln Cys Asn Gly Glu Val Asp Cys Ile Thr Gly Glu
        275                 280                 285

Asp Glu Val Gly Cys Ala Gly Phe Ala Ser Val Ala Gln Glu Glu Thr
    290                 295                 300

Glu Ile Leu Thr Ala Asp Met Asp Ala Glu Arg Arg Arg Ile Lys Ser
305                 310                 315                 320

Leu Leu Pro Lys Leu Ser Cys Gly Val Lys Asn Arg Met His Ile Arg
                325                 330                 335

Arg Lys Arg Ile Val Gly Gly Lys Arg Ala Gln Leu Gly Asp Leu Pro
            340                 345                 350

Trp Gln Val Ala Ile Lys Asp Ala Ser Gly Ile Thr Cys Gly Gly Ile
        355                 360                 365

Tyr Ile Gly Gly Cys Trp Ile Leu Thr Ala Ala His Cys Leu Arg Ala
    370                 375                 380

Ser Lys Thr His Arg Tyr Gln Ile Trp Thr Thr Val Val Asp Trp Ile
385                 390                 395                 400

His Pro Asp Leu Lys Arg Ile Val Ile Glu Tyr Val Asp Arg Ile Ile
                405                 410                 415

Phe His Glu Asn Tyr Asn Ala Gly Thr Tyr Gln Asn Asp Ile Ala Leu
            420                 425                 430

Ile Glu Met Lys Lys Asp Gly Asn Lys Lys Asp Cys Glu Leu Pro Arg
        435                 440                 445

Ser Ile Pro Ala Cys Val Pro Trp Ser Pro Tyr Leu Phe Gln Pro Asn
    450                 455                 460

Asp Thr Cys Ile Val Ser Gly Trp Gly Arg Glu Lys Asp Asn Glu Arg
465                 470                 475                 480
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```
Val Phe Ser Leu Gln Trp Gly Glu Val Lys Leu Ile Ser Asn Cys Ser
                485                 490                 495

Lys Phe Tyr Gly Asn Arg Phe Tyr Glu Lys Glu Met Glu Cys Ala Gly
            500                 505                 510

Thr Tyr Asp Gly Ser Ile Asp Ala Cys Lys Gly Asp Ser Gly Gly Pro
        515                 520                 525

Leu Val Cys Met Asp Ala Asn Asn Val Thr Tyr Val Trp Gly Val Val
    530                 535                 540

Ser Trp Gly Glu Asn Cys Gly Lys Pro Glu Phe Pro Gly Phe Tyr Thr
545                 550                 555                 560

Lys Val Ala Asn Tyr Phe Asp Trp Ile Ser Tyr His Val Gly Arg Pro
                565                 570                 575

Phe Ile Ser Gln Tyr Asn Val
            580


<210> SEQ ID NO 21
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

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

Glu Gly Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Asn Gly
            20                  25                  30

Gln Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys His Gln Tyr Asp Gly Ser Pro
                85                  90                  95

Glu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Asn Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Arg Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210> SEQ ID NO 22
<211> LENGTH: 396
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 22

```
Met Phe Leu Lys Ala Val Val Leu Thr Leu Ala Leu Val Ala Val Ala
1               5                   10                  15

Gly Ala Arg Ala Glu Val Ser Ala Asp Gln Val Ala Thr Val Met Trp
            20                  25                  30

Asp Tyr Phe Ser Gln Leu Ser Asn Asn Ala Lys Glu Ala Val Glu His
        35                  40                  45

Leu Gln Lys Ser Glu Leu Thr Gln Gln Leu Asn Ala Leu Phe Gln Asp
    50                  55                  60

Lys Leu Gly Glu Val Asn Thr Tyr Ala Gly Asp Leu Gln Lys Lys Leu
65                  70                  75                  80

Val Pro Phe Ala Thr Glu Leu His Glu Arg Leu Ala Lys Asp Ser Glu
                85                  90                  95

Lys Leu Lys Glu Glu Ile Gly Lys Glu Leu Glu Glu Leu Arg Ala Arg
            100                 105                 110

Leu Leu Pro His Ala Asn Glu Val Ser Gln Lys Ile Gly Asp Asn Leu
        115                 120                 125

Arg Glu Leu Gln Gln Arg Leu Glu Pro Tyr Ala Asp Gln Leu Arg Thr
    130                 135                 140

Gln Val Asn Thr Gln Ala Glu Gln Leu Arg Arg Gln Leu Thr Pro Tyr
145                 150                 155                 160

Ala Gln Arg Met Glu Arg Val Leu Arg Glu Asn Ala Asp Ser Leu Gln
                165                 170                 175

Ala Ser Leu Arg Pro His Ala Asp Glu Leu Lys Ala Lys Ile Asp Gln
            180                 185                 190

Asn Val Glu Glu Leu Lys Gly Arg Leu Thr Pro Tyr Ala Asp Glu Phe
        195                 200                 205

Lys Val Lys Ile Asp Gln Thr Val Glu Glu Leu Arg Arg Ser Leu Ala
    210                 215                 220

Pro Tyr Ala Gln Asp Thr Gln Glu Lys Leu Asn His Gln Leu Glu Gly
225                 230                 235                 240

Leu Thr Phe Gln Met Lys Lys Asn Ala Glu Glu Leu Lys Ala Arg Ile
                245                 250                 255

Ser Ala Ser Ala Glu Glu Leu Arg Gln Arg Leu Ala Pro Leu Ala Glu
            260                 265                 270

Asp Val Arg Gly Asn Leu Arg Gly Asn Thr Glu Gly Leu Gln Lys Ser
        275                 280                 285

Leu Ala Glu Leu Gly Gly His Leu Asp Gln Gln Val Glu Glu Phe Arg
    290                 295                 300

Arg Arg Val Glu Pro Tyr Gly Glu Asn Phe Asn Lys Ala Leu Val Gln
305                 310                 315                 320

Gln Met Glu Gln Leu Arg Gln Lys Leu Gly Pro His Ala Gly Asp Val
                325                 330                 335

Glu Gly His Leu Ser Phe Leu Glu Lys Asp Leu Arg Asp Lys Val Asn
            340                 345                 350

Ser Phe Phe Ser Thr Phe Lys Glu Lys Glu Ser Gln Asp Lys Thr Leu
        355                 360                 365
```

-continued

```
Ser Leu Pro Glu Leu Glu Gln Gln Gln Glu Gln Gln Gln Glu Gln Gln
    370                 375                 380

Gln Glu Gln Val Gln Met Leu Ala Pro Leu Glu Ser
385                 390                 395
```

What is claimed is:

1. A Method of use of protein biomarkers of neurodegenerative disease comprising two or more biomarkers in a biological sample, wherein the detection and/or the concentration of a first biomarker is employed to sort between categories of neurodegenerative disease patients and categories of normal and disease controls, and the presence and/or concentration of the first biomarker and of one or more additional biomarkers are then employed within each category for screening, diagnosis, differential diagnosis and monitoring of neurodegenerative disease severity and of disease mechanisms in the patients.

2. The method of claim **1** wherein the biological sample is blood.

3. The method of claim **2** wherein the blood sample is blood serum, or blood plasma, or whole blood, or blood cells.

4. The method of **1** wherein the biological sample is Cerebrospinal Fluid, urine, or tissue.

5. The method of claim **1** wherein the neurodegenerative disease is Alzheimer's disease (AD).

6. The method of claim **1** wherein the neurodegenerative disease is Parkinson's disease (PD).

7. The method of claim **1** wherein the neurodegenerative disease is Amyotrophic Lateral Sclerosis (ALS).

8. The method of claim **1**, wherein the biomarkers comprise two or more of proteins, such as an Apolipoprotein E4 protein, an Apolipoprotein E3 protein, an Apolipoprotein A-IV protein, a Transthyretin protein, A Complement Factor H protein, A Complement Factor Hs protein, a Complement Factor Bb protein, a Complement Factor I protein, a Complement C3c1 protein, a Complement C3c2a protein, a Complement C3dg protein, a Haptoglobin HP-1 protein, an Immunoglobulin Light Chain Protein, and/or an Inter-alpha Trypsin Inhibitor protein in a blood serum sample, for distinguishing between different categories of patients with Alzheimer's disease, and for screening, diagnosis, differential diagnosis and monitoring of Alzheimer's disease severity and disease mechanisms in the patients.

9. The method of claim **8**, for screening, diagnosis, differential diagnosis, and determining and monitoring of disease severity and mechanisms of Alzheimer's disease in patients, comprising:

obtaining a biological sample from a test subject;

determining whether or not a quantity of the first biomarker can be detected; and if so determining the quantity of that first biomarker in the biological sample; and

determining the quantities of one or more of the other biomarkers in the biological sample; and

determining the quantities of one or more additional biomarkers, in biological samples from normal control individuals, from patients with Alzheimer's disease, with Parkinson's disease, and with Alzheimer's disease-like (AD-like) and/or mixed disorders, wherein the detection of a quantity and/or the quantity of the first biomarker in the test subject biological sample is indicative of a particular form or variation of Alzheimer's disease or a normal condition with a potential to develop that particular form or variation of Alzheimer's disease, and the quantities of the first biomarker and of one or more additional biomarkers, in the biological sample of the test subject outside the range of that particular form or variation of Alzheimer's disease values are indicative of the absence of that form of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an Alzheimer's disease-like and/or mixed disorder, and wherein a lack of detection of a quantity and/or the quantity of the first biomarker in the test subject biological sample is indicative of another particular form or variation of Alzheimer's disease or a normal condition with a potential to develop that other particular form or variation of Alzheimer's disease, and the quantities of the first biomarker and of one or more other biomarkers in the biological sample of the test subject within the ranges of that other particular other form or variation of Alzheimer's disease values is indicative of the presence of that other particular form or variation of Alzheimer's disease, and the quantity of one or more other biomarkers, in the biological sample of the test subject outside the range of that other form of Alzheimer's disease values are indicative of the absence of that other particular form or variation of Alzheimer's disease and the presence of a normal condition, or another neurological disorder, such as Parkinson's disease, or an Alzheimer's disease-like or mixed disorder.

10. The Method of claim **9** wherein the Alzheimer's disease-like or mixed disorder is any one of a number of neurological disorders with symptoms similar to Alzheimer's disease, such as:

Frontotemporal dementia (FTD); Lewy body dementia (LBD); Corticalbasal Ganglionic degeneration, Alcohol related dementia; Semantic dementia; Vascular (Multi-infarct) dementia; Stroke (CVA); Post-irradiation Encephalopathy and Seizures; Alzheimer's disease combined with Vascular (Multi-Infarct) dementia; Alzheimer's disease combined with Lewy body dementia; Parkinson's disease combined with Lewy body dementia; Alzheimer's and Parkinson's disease combined with Lewy body dementia; Frontotemporal dementia combined with Chronic Inflammatory Demyelinating Polyneuropathy; and Thalamic CVA combined with HX of Lung CA, or Parkinson's disease or any of a number of other diseases where the disease causes symptoms similar to Alzheimer's disease.

11. The method of claim **1** wherein the detection and/or determination of quantities of biomarkers are performed by gel electrophoresis

12. The method of claim **11** wherein the detection and/or determination of quantities of biomarkers are performed by quantitative 2D gel electrophoresis.

13. The method of claim **1** wherein the detection and/or determination of quantities of biomarkers are performed by any form of immunoassay.

14. The method of claim **13** wherein the immunoassay is an ELISA assay.

15. The method of claim **14** wherein the immunoassay is an array of ELISA assays.

16. The method of claim **1** wherein the detection and/or determination of quantities of biomarkers are performed by Mass Spectrometry.

17. The method of claim **1** wherein the detection and/or determination of quantities of biomarkers are performed by chromatography

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专利名称(译)	基于血清中蛋白质生物标志物浓度差异的多种形式的阿尔茨海默病		
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摘要(译)

本发明涉及用于神经变性疾病（包括阿尔茨海默病）和相关疾病的生物标志物的鉴定和用途。更具体地，本发明涉及蛋白质生物标志物的鉴定，所述蛋白质生物标志物可用于从帕金森病，其他神经退行性疾病和正常对照中筛选，诊断和分化阿尔茨海默氏病，以及用于监测患者的阿尔茨海默氏病严重性和疾病机制。 。