US 20110165554A1

(19) **United States**

(12) **Patent Application Publication**

Levin et al.

(10) **Pub. No.: US 2011/0165554 A1**

(43) **Pub. Date: Jul. 7, 2011**

(54) **METHODS AND BIOMARKERS FOR DIAGNOSING AND MONITORING PSYCHOTIC DISORDERS**

(75) Inventors: **Yishai Levin**, Cambridge (GB); **Sabine Bahn**, Cambridge (GB); **Lan Wang**, Cambridge (GB); **Emanuel Schwarz**, Cambridge (GB); **George McAllister**, Cambridge (GB)

(73) Assignees: **Psynova Neurotech Limited**, Cambridge (GB); **Cambridge Enterprise Limited**, Cambridge (GB)

(21) Appl. No.: **12/809,269**

(22) PCT Filed: **Dec. 18, 2008**

(86) PCT No.: **PCT/GB2008/004186**

§ 371 (c)(1),
(2), (4) Date: **Mar. 3, 2011**

(30) **Foreign Application Priority Data**

Dec. 19, 2007 (GB) 0724735.6

Publication Classification

(51) **Int. Cl.**

C12Q 1/70	(2006.01)
C12Q 1/42	(2006.01)
C12Q 1/37	(2006.01)
C12Q 1/25	(2006.01)
G01N 33/53	(2006.01)
C12Q 1/02	(2006.01)
C12N 9/00	(2006.01)
G01R 33/46	(2006.01)
G01N 30/02	(2006.01)
G01N 21/33	(2006.01)
G01J 3/46	(2006.01)
G01N 33/48	(2006.01)
H01J 49/26	(2006.01)

(52) **U.S. Cl.** **435/5**; 435/21; 435/23; 435/4; 435/7.92; 436/501; 435/29; 435/183; 324/307; 73/61.52; 356/51; 356/402; 73/61.43; 250/282

(57) **ABSTRACT**

A method of diagnosing or monitoring a psychotic disorder, or predisposition thereto, comprises measuring, in a sample taken from a subject, the levels of one or more peptide biomarkers listed herein.

METHODS AND BIOMARKERS FOR DIAGNOSING AND MONITORING PSYCHOTIC DISORDERS

TECHNICAL FIELD

[0001] The present invention relates to methods of diagnosing or of monitoring psychotic disorders, in particular schizophrenic disorders (and bipolar disorders), e.g. using biomarkers. The invention also relates to use of biomarkers in clinical screening, assessment of prognosis, evaluation of therapy, drug screening and drug development. The biomarkers and methods in which they are employed can be used to assist diagnosis, and to assess onset and development of psychotic disorders.

BACKGROUND OF THE INVENTION

[0002] The identification of biomarkers for schizophrenic disorders permits integration of diagnostic procedures and therapeutic regimes. Currently, there are significant delays in determining effective treatment and it has not hitherto been possible to perform rapid assessment of drug response. Traditionally, many anti-schizophrenic therapies have required treatment trials lasting weeks to months for a given therapeutic approach.

[0003] Yang et al (2006), Anal. Chem. 78, 3571-6, discloses altered levels of proteins in the plasma of patients with schizophrenia. The results relate to markers of drug efficacy. There is apparently no difference between treated and non-treated patients. No quantitative results are given.

[0004] WO2007/045865 (the content of which is incorporated by reference) describes psychosis and other disorders and the need for biomarkers. The biomarkers described there include ApoA1 (apolipoprotein) peptide.

[0005] WO2008/090319 (unpublished at the priority date claimed herein; the content of which is also incorporated by reference) discloses further biomarkers. They include clusterin precursor, inter alpha-trypsin inhibitor, apolipoprotein A2 and alpha2 HS glycoprotein.

SUMMARY OF THE INVENTION

[0006] Based on an approach of the type described in WO2007/045865, further biomarkers have been identified. According to one aspect of the invention, a method of diagnosing or monitoring a psychotic disorder, or predisposition thereto, comprises monitoring the level of one or more biomarkers present in a sample taken from a subject, the biomarkers including at least one defined herein.

[0007] Further aspects of the invention are defined in the claims, and/or are the same procedures/products as described for biomarkers in WO2006/045865. They include a method for monitoring efficacy of therapy for a schizophrenic disorder in a subject. Monitoring methods of the invention can be used to monitor onset, progression, stabilisation, amelioration and/or remission of a psychotic disorder.

[0008] Another aspect of the invention is a method of identifying a substance capable of stimulating, promoting or activating the generation of a peptide biomarker in a subject, comprising administering a test substance to a subject animal and detecting and/or quantifying levels of the peptide biomarker present in a test sample from the subject. Further aspects of the invention are the use of a substance or ligand according to the invention in the treatment of a schizophrenic disorder or predisposition thereto and as a medicament. The substance may be used according to the invention in the manufacture of a medicament for the treatment of a schizophrenic disorder or predisposition thereto.

DESCRIPTION OF THE INVENTION

[0009] In use of the invention, it will be appreciated that a single biomarker or more than one may be used, on one or more than one occasion, and with respect to one or more samples. Reference may be made to protein biomarkers, and it will be appreciated that, according to circumstances, reference to such a protein includes fragments thereof.

[0010] In testing, according to the invention, a changed or lower level of plasma protein biomarkers in a test biological sample relative to the level in a normal control is indicative of the presence of a psychotic disorder, in particular a schizophrenic disorder, bipolar disorder, or predisposition thereto. A decrease in the level of plasma protein in a biological sample, preferably in a sample of whole blood, plasma, or serum over time may be indicative of onset or progression, i.e. worsening of the disorder, whereas an increase in the level of plasma protein may indicate amelioration or remission of the disorder.

[0011] Methods of monitoring and of diagnosis according to the invention are useful to confirm the existence of a disorder, or predisposition thereto, to monitor development of the disorder by assessing onset and progression, or to assess amelioration or regression of the disorder. Methods of monitoring and of diagnosis are also useful in methods for assessment of clinical screening, prognosis, choice of therapy, evaluation of therapeutic benefit, i.e. for drug screening and drug development.

[0012] Efficient diagnosis and monitoring methods provide very powerful "patient solutions" with the potential for improved prognosis, by establishing the correct diagnosis, allowing rapid identification of the most appropriate treatment (thus lessening unnecessary exposure to harmful drug side effects), reducing "down-time" and relapse rates.

[0013] Methods for monitoring efficacy of a therapy can be used to monitor the therapeutic effectiveness of existing therapies and new therapies in human subjects and in non-human animals (e.g. in animal models). These monitoring methods can be incorporated into screens for new drug substances and combinations of substances.

[0014] Modulation of a peptide biomarker level is useful as an indicator of the state of the schizophrenic disorder or predisposition thereto. A decrease in the level of peptide biomarker over time is indicative of onset or progression, i.e. worsening of the disorder, whereas an increase in the level of peptide biomarker indicates amelioration or remission of the disorder.

[0015] Detection of a peptide biomarker of the invention can be used to screen subjects prior to their participation in clinical trials. The biomarker provides a means to indicate therapeutic response, failure to respond, unfavourable side-effect profile, degree of medication compliance and achievement of adequate serum drug levels. The biomarker may be used to provide warning of adverse drug response, a major problem encountered with all psychotropic medications. Biomarkers are useful in development of personalized brain therapies, as assessment of response can be used to fine-tune dosage, minimise the number of prescribed medications, reduce the delay in attaining effective therapy and avoid adverse drug reactions. Thus, by monitoring a biomarker of the invention, patient care can be tailored precisely to match

the needs determined by the disorder and the pharmacogenomic profile of the patient; the biomarker can thus be used to titrate the optimal dose, predict a positive therapeutic response, and identify those patients at high risk of severe side effects.

[0016] Biomarker-based tests provide a first-line assessment of 'new' patients, and provide objective measures for accurate and rapid diagnosis, in a time frame and with precision, not achievable using the current subjective measures.

[0017] Furthermore, diagnostic biomarker tests are useful to identify family members or patients in the "prodromal phase", i.e. those at high risk of developing overt schizophrenia. This permits initiation of appropriate therapy, for example low dose antipsychotics, or preventive measures, e.g. managing risk factors such as stress, illicit drug use or viral infections. These approaches are recognised to improve outcome and may prevent overt onset of the disorder.

[0018] Biomarker monitoring methods, biosensors and kits are also vital as patient monitoring tools, to enable the physician to determine whether relapse is due to a genuine breakthrough or worsening of the disease, poor patient compliance or substance abuse. If pharmacological treatment is assessed to be inadequate, then therapy can be reinstated or increased. For genuine breakthrough disease, a change in therapy can be given if appropriate. As the biomarker is sensitive to the state of the disorder, it provides an indication of the impact of drug therapy or of substance abuse.

[0019] High-throughput screening technologies based on the biomarkers of the invention, uses and methods of the invention, e.g. configured in an array format, are suitable to monitor biomarkers for the identification of potentially useful therapeutic compounds, e.g. ligands such as natural compounds, synthetic chemical compounds (e.g. from combinatorial libraries), peptides, monoclonal or polyclonal antibodies or fragments thereof, capable of modulating the expression of the biomarkers.

[0020] The term "biomarker" means a distinctive biological or biologically derived indicator of a process, event or condition. Peptide biomarkers can be used in methods of diagnosis, e.g. clinical screening, and prognosis assessment; and in monitoring the results of therapy, for identifying patients most likely to respond to a particular therapeutic treatment, as well as in drug screening and development. Biomarkers and uses thereof are valuable for identification of new drug treatments and for discovery of new targets for drug treatment.

[0021] The term "drug-naïve patient" as used herein means an individual who has not been treated with any schizophrenia therapeutic substance. In a preferred embodiment, the invention relates to a method wherein the test sample is from a test subject wherein the test subject is a first onset drug-naïve individual, and the sample is taken prior to administration of any anti-schizophrenic therapy to the subject. The control sample is preferably a sample from a normal individual.

[0022] The term "diagnosis" as used herein encompasses identification, confirmation, and/or characterisation of a schizophrenic disorder or predisposition thereto. The term "predisposition" as used herein means that a subject does not currently present with the disorder, but is liable to be affected by the disorder in time. Methods of diagnosis according to the invention are useful to confirm the existence of a disorder, or predisposition thereto. Methods of diagnosis are also useful in methods for assessment of clinical screening, prognosis, choice of therapy, evaluation of therapeutic benefit, i.e. for drug screening and drug development.

[0023] The term "psychotic disorder" as used herein refers to a disorder in which psychosis is a recognised symptom, this includes neuropsychiatric (psychotic depression and other psychotic episodes) and neurodevelopmental disorders (especially Autistic spectrum disorders), neurodegenerative disorders, depression, mania, and in particular, schizophrenic disorders (paranoid, catatonic, disorganized, undifferentiated and residual schizophrenia) and bipolar disorders.

[0024] Biological samples that may be tested in a method of the invention include whole blood, blood serum or plasma, urine, saliva, cerebrospinal fluid (CSF) or other bodily fluid (stool, tear fluid, synovial fluid, sputum), breath, e.g. as condensed breath, or an extract or purification therefrom, or dilution thereof. Biological samples also include tissue homogenates, tissue sections and biopsy specimens from a live subject, or taken post-mortem. The samples can be prepared, for example where appropriate diluted or concentrated, and stored in the usual manner.

[0025] A number of spectroscopic techniques can be used to generate spectra, according to the invention, including NMR spectroscopy and mass spectrometry. In preferred methods, spectral analysis is performed by NMR spectroscopy, preferably ^{1}H NMR spectroscopy. One or more spectra may be generated; a suite of spectra may be measured, including one for small molecules and another for macromolecule profiles. The spectra obtained may be subjected to spectral editing techniques. One or two-dimensional NMR spectroscopy may be performed.

[0026] An advantage of using NMR spectroscopy to study complex biomixtures is that measurements can often be made with minimal sample preparation (usually with only the addition of 5-10% D_2O) and a detailed analytical profile of the whole biological sample can be obtained.

[0027] Sample volumes are small, typically 0.3 to 0.5 ml for standard probes, and as low as 3 μl for microprobes. Acquisition of simple NMR spectra is rapid and efficient using flow-injection technology. It is usually necessary to suppress the water NMR resonance.

[0028] High resolution NMR spectroscopy (in particular ^{1}H NMR) is particularly appropriate. The main advantages of using ^{1}H NMR spectroscopy are the speed of the method (with spectra being obtained in 5 to 10 minutes), the requirement for minimal sample preparation, and the fact that it provides a non-selective detector for all metabolites in the biofluid regardless of their structural type, provided only that they are present above the detection limit of the NMR experiment and that they contain non-exchangeable hydrogen atoms.

[0029] NMR studies of body fluids should ideally be performed at the highest magnetic field available to obtain maximal dispersion and sensitivity and most ^{1}H NMR studies are performed at 400 MHz or greater, e.g. 600 MHz.

[0030] Usually, to assign ^{1}H NMR spectra, comparison is made with control spectra of authentic materials and/or by standard addition of an authentic reference standard to the sample. The control spectra employed may be normal control spectra, generated by spectral analysis of a biological sample from a normal subject, and/or psychotic disorder control spectra, generated by spectral analysis of a biological sample from a subject with a psychotic disorder.

[0031] Additional confirmation of assignments is usually sought from the application of other NMR methods, includ-

ing, for example, 2-dimensional (2D) NMR methods, particularly COSY (correlation spectroscopy), TOCSY (total correlation spectroscopy), inverse-detected heteronuclear correlation methods such as HMBC (heteronuclear multiple bond correlation), HSQC (heteronuclear single quantum coherence), and HMQC (heteronuclear multiple quantum coherence), 2D J-resolved (JRES) methods, spin-echo methods, relaxation editing, diffusion editing (e.g., both 1D NMR and 2D NMR such as diffusion-edited TOCSY), and multiple quantum filtering.

[0032] By comparison of spectra with normal and/or psychotic disorder control spectra, the test spectra can be classified as having a normal profile, a psychotic disorder profile, or a psychotic disorder predisposition profile.

[0033] Comparison of spectra may be performed on entire spectra or on selected regions of spectra. Comparison of spectra may involve an assessment of the variation in spectral regions responsible for deviation from the normal spectral profile and in particular, assessment of variation in one or more biomarkers within those regions.

[0034] A limiting factor in understanding the biochemical information from both 1D and 2D-NMR spectra of biofluids, such as plasma, is their complexity. The most efficient way to compare and investigate these complex multiparametric data is employ the 1D or 2D NMR metabonomic approach in combination with computer-based "pattern recognition" (PR) methods and expert systems.

[0035] Although the utility of the metabonomic approach is well established, its full potential has not yet been exploited. The metabolic variation is often subtle, and powerful analysis methods are required for detection of particular analytes, especially when the data (e.g., NMR spectra) are so complex.

[0036] Metabonomics methods (which employ multivariate statistical analysis and pattern recognition (PR) techniques, and optionally data filtering techniques) of analysing data (e.g. NMR spectra) from a test population yield accurate mathematical models which may subsequently be used to classify a test sample or subject, and/or in diagnosis.

[0037] Comparison of spectra may include one or more chemometric analyses of the spectra. The term "chemometrics" is applied to describe the use of pattern recognition (PR) methods and related multivariate statistical approaches to chemical numerical data. Comparison may therefore comprise one or more pattern recognition analysis methods, which can be performed by one or more supervised and/or unsupervised methods.

[0038] Pattern recognition (PR) methods can be used to reduce the complexity of data sets, to generate scientific hypotheses and to test hypotheses. In general, the use of pattern recognition algorithms allows the identification, and, with some methods, the interpretation of some non-random behaviour in a complex system which can be obscured by noise or random variations in the parameters defining the system. Also, the number of parameters used can be very large such that visualisation of the regularities or irregularities, which for the human brain is best in no more than three dimensions, can be difficult.

[0039] Usually the number of measured descriptors is much greater than three and so simple scatter plots cannot be used to visualise any similarity or disparity between samples. Pattern recognition methods have been used widely to characterise many different types of problem ranging for example over linguistics, fingerprinting, chemistry and psychology.

[0040] In the context of the methods described herein, pattern recognition is the use of multivariate statistics, both parametric and non-parametric, to analyse spectroscopic data, and hence to classify samples and to predict the value of some dependent variable based on a range of observed measurements. There are two main approaches. One set of methods is termed "unsupervised" and these simply reduce data complexity in a rational way and also produce display plots which can be interpreted by the human eye. The other approach is termed "supervised" whereby a training set of samples with known class or outcome is used to produce a mathematical model and this is then evaluated with independent validation data sets.

[0041] Unsupervised techniques are used to establish whether any intrinsic clustering exists within a data set and consist of methods that map samples, often by dimension reduction, according to their properties, without reference to any other independent knowledge, e.g. without prior knowledge of sample class. Examples of unsupervised methods include principal component analysis (PCA), non-linear mapping (NLM) and clustering methods such as hierarchical cluster analysis.

[0042] One of the most useful and easily applied unsupervised PR techniques is principal components analysis (PCA) (see, for example, Kowalski et al., 1986). Principal components (PCs) are new variables created from linear combinations of the starting variables with appropriate weighting coefficients. The properties of these PCs are such that: (i) each PC is orthogonal to (uncorrelated with) all other PCs, and (ii) the first PC contains the largest part of the variance of the data set (information content) with subsequent PCs containing correspondingly smaller amounts of variance.

[0043] PCA, a dimension reduction technique, takes m objects or samples, each described by values in K dimensions (descriptor vectors), and extracts a set of eigenvectors, which are linear combinations of the descriptor vectors. The eigenvectors and eigen values are obtained by diagonalisation of the covariance matrix of the data. The eigenvectors can be thought of as a new set of orthogonal plotting axes, called principal components (PCs). The extraction of the systematic variations in the data is accomplished by projection and modelling of variance and covariance structure of the data matrix. The primary axis is a single eigenvector describing the largest variation in the data, and is termed principal component one (PC1). Subsequent PCs, ranked by decreasing eigen value, describe successively less variability. The variation in the data that has not been described by the PCs is called residual variance and signifies how well the model fits the data. The projections of the descriptor vectors onto the PCs are defined as scores, which reveal the relationships between the samples or objects. In a graphical representation (a "scores plot" or eigenvector projection), objects or samples having similar descriptor vectors will group together in clusters. Another graphical representation is called a loadings plot, and this connects the PCs to the individual descriptor vectors, and displays both the importance of each descriptor vector to the interpretation of a PC and the relationship among descriptor vectors in that PC. In fact, a loading value is simply the cosine of the angle which the original descriptor vector makes with the PC.

[0044] Descriptor vectors which fall close to the origin in this plot carry little information in the PC, while descriptor vectors distant from the origin (high loading) are important for interpretation. Thus, a plot of the first two or three PC

scores gives the "best" representation, in terms of information content, of the data set in two or three dimensions, respectively. A plot of the first two principal component scores, PC1 and PC2, provides the maximum information content of the data in two dimensions. Such PC maps can be used to visualise inherent clustering behaviour, for example, for drugs and toxins based on similarity of their metabonomic responses and hence mechanism of action. Of course, the clustering information may be in lower PCs and these can also be examined.

[0045] Hierarchical Cluster Analysis, another unsupervised pattern recognition method, permits the grouping of data points which are similar by virtue of being "near" to one another in some multidimensional space. Individual data points may be, for example, the signal intensities for particular assigned peaks in an NMR spectrum. A "similarity matrix" S, is constructed with element ssij=1−rij/rijmax' where rij is the interpoint distance between points i and j (e.g., Euclidean interpoint distance), and rijmax is the largest interpoint distance for all points.

[0046] The most distant pair of points will have sij equal to 0, since rij then equals rijmaX. Conversely, the closest pair of points will have the largest sij, approaching 1. The similarity matrix is scanned for the closest pair of points. The pair of points is reported with their separation distance, and then the two points are deleted and replaced with a single combined point. The process is then repeated iteratively until only one point remains. A number of different methods may be used to determine how two clusters will be joined, including the nearest neighbour method (also known as the single link method), the furthest neighbour method, the centroid method (including centroid link, incremental link, median link, group average link, and flexible link variations).

[0047] The reported connectivities are then plotted as a dendrogram (a tree-like chart which allows visualisation of clustering), showing sample-sample connectivities versus increasing separation distance (or equivalently, versus decreasing similarity). In the dendrogram the branch lengths are proportional to the distances between the various clusters and hence the length of the branches linking one sample to the next is a measure of their similarity. In this way, similar data points may be identified algorithmically.

[0048] Supervised methods of analysis use the class information given for a training set of sample data to optimise the separation between two or more sample classes. These techniques include soft independent modelling of class analogy, partial least squares (PLS) methods, such as projection to latent discriminant analysis (PLS DA), k-nearest neighbour analysis and neural networks. Neural networks are a non-linear method of modelling data. A training set of data is used to develop algorithms that 'learn' the structure of the data and can cope with complex functions. Several types of neural network have been applied successfully to predicting toxicity or disease from spectral information.

[0049] Statistical techniques such as one-way analysis of variance (ANOVA) may also be employed to analyse data.

[0050] Methods of the invention involving spectral analysis may be performed to provide spectra from biological samples taken on two or more occasions from a test subject. Spectra from biological samples taken on two or more occasions from a test subject can be compared to identify differences between the spectra of samples taken on different occasions. Methods may include analysis of spectra from biological samples taken on two or more occasions from a test subject to quantify the level of one or more biomarkers present in the biological samples, and comparing the level of the one or more biomarkers present in biological samples taken on two or more occasions.

[0051] Diagnostic and monitoring methods of the invention are useful in methods of assessing prognosis of a psychotic disorder, in methods of monitoring efficacy of an administered therapeutic substance in a subject having, suspected of having, or of being predisposed to, a psychotic disorder and in methods of identifying an anti-psychotic or pro-psychotic substance. Such methods may comprise comparing the level of the one or more biomarkers in a test biological sample taken from a test subject with the level present in one or more samples taken from the test subject prior to administration of the substance, and/or one or more samples taken from the test subject at an earlier stage during treatment with the substance. Additionally, these methods may comprise detecting a change in the level of the one or more biomarkers in biological samples taken from a test subject on two or more occasions.

[0052] In methods of the invention, in particular those in which spectral analysis is employed, and in particular when the biological sample is blood or is derived from blood, e.g. plasma or serum, a suitable biomarker is as listed herein.

[0053] A method according to the invention may comprise comparing the level of one or more biomarkers in a biological sample taken from a test subject with the level present in one or more samples taken from the test subject prior to commencement of a therapy, and/or one or more samples taken from the test subject at an earlier stage of a therapy. Such methods may comprise detecting a change in the amount of the one or more biomarkers in samples taken on two or more occasions. Methods of the invention are particularly useful in assessment of anti-psychotic therapies.

[0054] A method of diagnosis of or monitoring according to the invention may comprise quantifying the one or more biomarkers in a test biological sample taken from a test subject and comparing the level of the one or more biomarkers present in said test sample with one or more controls. The control can be selected from a normal control and/or a psychotic disorder control. The control used in a method of the invention can be one or more controls selected from the group consisting of: the level of biomarker found in a normal control sample from a normal subject, a normal biomarker level; a normal biomarker range, the level in a sample from a subject with a schizophrenic disorder, bipolar disorder, related psychotic disorder, or a diagnosed predisposition thereto; a schizophrenic disorder marker level, a bipolar disorder marker level, a related psychotic disorder marker level, a schizophrenic disorder marker range, a bipolar disorder marker range and a related psychotic disorder marker range.

[0055] Biological samples can be taken at intervals over the remaining life, or a part thereof, of a subject. Suitably, the time elapsed between taking samples from a subject undergoing diagnosis or monitoring will be 3 days, 5 days, a week, two weeks, a month, 2 months, 3 months, 6 or 12 months. Samples may be taken prior to and/or during and/or following an anti-psychotic therapy, such as an anti-schizophrenic or anti-bipolar disorder therapy.

[0056] Measurement of the level of a biomarker can be performed by any method suitable to identify the amount of the biomarker in a biological sample taken from a patient or a purification of or extract from the sample or a dilution thereof. Measuring the level of a biomarker present in a sample may

include determining the concentration of the biomarker present in the sample. Such quantification may be performed directly on the sample, or indirectly on an extract therefrom, or on a dilution thereof. In methods of the invention, in addition to measuring the concentration of the biomarker in a biological sample, which is preferably whole blood, plasma or serum, the concentration of the biomarker may be tested in a different biological sample taken from the test subject, e.g. CSF, urine, saliva, or other bodily fluid (stool, tear fluid, synovial fluid, sputum), breath, e.g. as condensed breath, or an extract or purification therefrom, or dilution thereof. Biological samples also include tissue homogenates, tissue sections and biopsy specimens from a live subject, or taken post-mortem. The samples can be prepared, for example where appropriate diluted or concentrated, and stored in the usual manner.

[0057] Biomarker levels can be measured by one or more methods selected from the group consisting of: spectroscopy methods such as NMR (nuclear magnetic resonance), or mass spectroscopy (MS); SELDI (-TOF), MALDI (-TOF), a 1-D gel-based analysis, a 2-D gel-based analysis, liquid chromatography (e.g. high pressure liquid chromatography (HPLC) or low pressure liquid chromatography (LPLC)), thin-layer chromatography, and LC-MS-based techniques. Appropriate LC MS techniques include ICAT® (Applied Biosystems, CA, USA), or iTRAQ® (Applied Biosystems, CA, USA).

[0058] Measurement of a biomarker may be performed by a direct or indirect detection method. A biomarker may be detected directly, or indirectly, via interaction with a ligand or ligands, such as an enzyme, binding receptor or transporter protein, antibody, peptide, aptamer, or oligonucleotide, or any synthetic chemical receptor or compound capable of specifically binding the biomarker. The ligand may possess a detectable label, such as a luminescent, fluorescent or radioactive label and/or an affinity tag.

[0059] The term "antibody" as used herein includes, but is not limited to: polyclonal, monoclonal, bispecific, humanised or chimeric antibodies, single chain antibodies, Fab fragments and F $(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein also refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

[0060] Metabolite biomarkers as described herein are suitably measured by conventional chemical or enzymatic methods (which may be direct or indirect and or may not be coupled), electrochemical, fluorimetric, luminometric, spectrophotometric, fluorimetric, luminometric, spectrometric, polarimetric, chromatographic (e.g. HPLC) or similar techniques.

[0061] For enzymatic methods, consumption of a substrate in the reaction, or generation of a product of the reaction, may be detected, directly or indirectly, as a means of measurement.

[0062] The biomarkers of the invention are preferably detected and measured using mass spectrometry-based techniques; chromatography-based techniques; enzymatic detection systems (by direct or indirect measurements); or using sensors, e.g. with sensor systems with amperometric, potentiometric, conductimetric, impedance, magnetic, optical, acoustic or thermal transducers.

[0063] A sensor may incorporate a physical, chemical or biological detection system. An example of a sensor is a biosensor, i.e. a sensor with a biological recognition system, e.g. based on a nucleic acid, such as an oligonucleotide probe or aptamer, or a protein such as an enzyme, binding protein, receptor protein, transporter protein or antibody.

[0064] The biosensor may incorporate an immunological method for detection of the biomarker, an electrical, thermal, magnetic, optical (e.g. hologram) or acoustic technologies. Using such biosensors, it is possible to detect the target biomarker at the anticipated concentrations found in biological samples.

[0065] Methods of the invention are suitable for clinical screening, assessment of prognosis, monitoring the results of therapy, identifying patients most likely to respond to a particular therapeutic treatment, for drug screening and development, and to assist in identification of new targets for drug treatment. The identification of key biomarkers specific to a disease is central to integration of diagnostic procedures and therapeutic regimes.

[0066] Methods of the invention may further comprise one or more assessments to diagnose and/or monitor a psychotic disorder in a subject. Assessment may be a clinical assessment, carried out by a clinician in accordance with accepted assessment protocols, e.g. global functioning score (GAF) or SCID, and/or may involve a self-assessment by the subject. Rating scales may be used to assist diagnosis and/or monitoring. GAF and SCID are assessed on the basis of a clinical interview. It is preferred that assessments, such as global functioning score, are made at (i.e. the same day as) or around (i.e. within a few days of) the time of collection of the test biological sample from the subject. This is particularly useful as a tool for diagnosing and monitoring female subjects, in which VLDL and LDL levels were found to have a very close inverse correlation with the clinical assessment as determined by global functioning score.

[0067] Using predictive biomarkers such as those described herein, appropriate diagnostic tools such as sensors and biosensors can be developed, accordingly, in methods and uses of the invention, detecting and quantifying one or more biomarkers can be performed using a sensor or biosensor.

[0068] The sensor or biosensor may incorporate detection methods and systems as described herein for detection of the biomarker. Sensors or biosensors may employ electrical (e.g. amperometric, potentiometric, conductimetric, or impedance detection systems), thermal (e.g. transducers), magnetic, optical (e.g. hologram) or acoustic technologies. In a sensor or biosensor according to the invention the level of one, two, or three biomarkers can be detected by one or more methods selected from: direct, indirect or coupled enzymatic, spectrophotometric, fluorimetric, luminometric, spectrometric, polarimetric and chromatographic techniques. Particularly preferred sensors or biosensors comprise one or more enzymes used directly or indirectly via a mediator, or using a binding, receptor or transporter protein, coupled to an electrical, optical, acoustic, magnetic or thermal transducer. Using such biosensors, it is possible to detect the level of target biomarkers at the anticipated concentrations found in biological samples.

[0069] A biomarker of the invention can be detected using a sensor or biosensor incorporating technologies based on

"smart" holograms, or high frequency acoustic systems, such systems are particularly amenable to "bar code" or array configurations. In smart hologram sensors (Smart Holograms Ltd, Cambridge, UK), a holographic image is stored in a thin polymer film that is sensitised to react specifically with the biomarker. On exposure, the biomarker reacts with the polymer leading to an alteration in the image displayed by the hologram. The test result read-out can be a change in the optical brightness, image, colour and/or position of the image. For qualitative and semi-quantitative applications, a sensor hologram can be read by eye, thus removing the need for detection equipment. A simple colour sensor can be used to read the signal when quantitative measurements are required. Opacity or colour of the sample does not interfere with operation of the sensor. The format of the sensor allows multiplexing for simultaneous detection of several substances. Reversible and irreversible sensors can be designed to meet different requirements, and continuous monitoring of a particular biomarker of interest is feasible.

[0070] Suitably, biosensors for detection of the biomarker of the invention are coupled, i.e. they combine biomolecular recognition with appropriate means to convert detection of the presence, or quantitation, of the biomarker in the sample into a signal. Biosensors can be adapted for "alternate site" diagnostic testing, e.g. in the ward, outpatients' department, surgery, home, field and workplace.

[0071] Biosensors to detect the biomarker of the invention include acoustic, plasmon resonance, holographic and microengineered sensors. Imprinted recognition elements, thin film transistor technology, magnetic acoustic resonator devices and other novel acousto-electrical systems may be employed in biosensors for detection of the biomarkers of the invention.

[0072] Methods involving detection and/or quantification of the biomarker of the invention can be performed using bench-top instruments, or can be incorporated onto disposable, diagnostic or monitoring platforms that can be used in a non-laboratory environment, e.g. in the physician's office or at the patient's bedside. Suitable sensors or biosensors for performing methods of the invention include "credit" cards with optical or acoustic readers. Sensors or biosensors can be configured to allow the data collected to be electronically transmitted to the physician for interpretation and thus can form the basis for e-neuromedicine.

[0073] Methods for monitoring efficacy of a therapy can be used to monitor the therapeutic effectiveness of existing therapies and new therapies in human subjects and in non-human animals (e.g. in animal models). These monitoring methods can be incorporated into screens for new drug substances and combinations of substances.

[0074] An increase in the level of the peptide biomarker in the test sample relative to the level in a previous test sample taken earlier from the same test subject is indicative of a beneficial effect, e.g. stabilisation or improvement, of said therapy on the disorder, suspected disorder or predisposition thereto.

[0075] Suitably, the time elapsed between taking samples from a subject undergoing diagnosis or monitoring will be 3 days, 5 days, a week, two weeks, a month, 2 months, 3 months, 6 or 12 months. Samples may be taken prior to and/or during and/or following an anti-schizophrenic disorder therapy. Samples can be taken at intervals over the remaining life, or a part thereof, of a subject.

[0076] Quantifying the amount of the biomarker present in a sample may include determining the concentration of the peptide biomarker present in the sample. Detecting and/or quantifying may be performed directly on the sample, or indirectly on an extract therefrom, or on a dilution thereof.

[0077] Detecting and/or quantifying can be performed by any method suitable to identify the presence and/or amount of a specific protein in a biological sample from a patient or a purification of extract of a biological sample or a dilution thereof. In methods of the invention, quantifying may be performed by measuring the concentration of the peptide biomarker in the sample or samples. Biological samples that may be tested in a method of the invention include cerebrospinal fluid (CSF), whole blood, blood serum, urine, saliva, or other bodily fluid (stool, tear fluid, synovial fluid, sputum), breath, e.g. as condensed breath, or an extract or purification therefrom, or dilution thereof. Biological samples also include tissue homogenates, tissue sections and biopsy specimens from a live subject, or taken post-mortem. Preferably, the sample is CSF or blood serum. The samples can be prepared, for example where appropriate diluted or concentrated, and stored in the usual manner.

[0078] Detection and/or quantification of peptide biomarkers may be performed by detection of the peptide biomarker or of a fragment thereof, e.g. a fragment with C-terminal truncation, and/or with N-terminal truncation. Fragments are suitably greater than 4 amino acids in length. Preferably, fragments are in the range of from about 6 to about 50 amino acids in length.

[0079] The biomarker may be directly detected, e.g. by SELDI or MALDI-TOF. Alternatively, the biomarker may be detected, directly or indirectly, via interaction with a ligand or ligands such as an antibody or a biomarker-binding fragment thereof, or other peptide, or ligand, e.g. aptamer, or oligonucleotide, capable of specifically binding the biomarker. The ligand may possess a detectable label, such as a luminescent, fluorescent or radioactive label, and/or an affinity tag. Ligands include, for example:

(1) in vivo: T3, T4 (thyroid hormones), vitamin A (indirectly by interacting with serum retinol-binding protein), apolipoprotein Al (ApoAl), noradrenaline oxidation products, and pterins.

(2) in vitro (most of them pharmacological agents): some non-steroidal anti-inflammatory drugs (NSAIDs), environmental pollutants, such as polyhalogenated biphenyls and thyromimetic compounds, xanthone derivatives as well as natural and synthetic flavonoids.

[0080] For example, methods relating to detecting, monitoring, diagnosing and/or quantifying can be performed by one or more methods selected from the group consisting of: SELDI (-TOF), MALDI (-TOF), a 1-D gel-based analysis, a 2-D gel-based analysis, Mass spec (MS), LC and LC-MS-based techniques. Appropriate LC MS techniques include ICAT® (Applied Biosystems, CA, USA), or iTRAQ® (Applied Biosystems, CA, USA). Liquid chromatography (e.g. high pressure liquid chromatography (HPLC) or low pressure liquid chromatography (LPLC)), thin-layer chromatography, NMR (nuclear magnetic resonance) spectroscopy could also be used.

[0081] Methods for diagnosis or monitoring according to the invention may comprise analysing a biological sample, e.g. cerebrospinal fluid (CSF) or serum, by SELDI-TOF, MALDI-TOF and other methods using mass spectrometry to detect the presence or level of the peptide biomarker. Such

techniques may be used for relative and absolute quantification and also to assess the ratio of the biomarker according to the invention with other biomarkers that may be present. These methods are also suitable for clinical screening, prognosis, monitoring the results of therapy, identifying patients most likely to respond to a particular therapeutic treatment, for drug screening and development, and identification of new targets for drug treatment.

[0082] Surface-enhanced laser deionization ionization (SELDI) mass spectrometry is a powerful tool for identifying a characteristic "fingerprint" of proteins and peptides in body fluids and tissues for a given condition, e.g. drug treatments and diseases. This technology utilizes protein chips to capture proteins/peptides and a time-of-flight mass spectrometer (tof-MS) to quantitate and calculate the mass of compounds ranging from small molecules and peptides of less than 1,000 Da up to proteins of 500 kDa. Quantifiable differences in protein/peptide patterns can be statistically evaluated using automated computer programs which represent each protein/peptide measured in the biofluid spectrum as a coordinate in multi-dimensional space. This approach has been most successful in the field of clinical biomarker discovery as it can be used as a diagnostic tool without knowing the biomarkers' identity. The SELDI system also has a capability of running hundreds of samples in a single experiment. In addition, all the signals from SELDI mass spectrometry are derived from native proteins/peptides (unlike some other proteomics technologies which require protease digestion), thus directly reflecting the underlying physiology of a given condition.

[0083] Detecting and/or quantifying the peptide biomarker may be performed using any method based on immunological, peptide, aptamer or synthetic recognition. For example, the method may involve an antibody, or a fragment thereof capable of specific binding to the peptide biomarker.

[0084] Any suitable animal may be used as a subject. It may be a human or non-human animal, for example a non-human primate, horse, cow, pig, goat, zebrafish, sheep, dog, cat, fish, rodent, e.g. guinea pig, rat or mouse, an insect (e.g. *Drosophila*), amphibian (e.g. *Xenopus*) or *C. elegans*.

[0085] When used in a method of identification, the test substance can be a known chemical or pharmaceutical substance, such as, but not limited to, an anti-schizophrenic disorder therapeutic, or a synthetic or natural chemical entity, or a combination of two or more of the aforesaid substances.

[0086] Identifying a substance capable of stimulating, promoting or activating the generation of a peptide biomarker, in a subject, may comprise exposing a test cell to a test substance and monitoring levels of the peptide biomarker within said test cell, or secreted by said test cell. The test cell could be prokaryotic, however it is preferred that a eukaryotic cell be employed in cell-based testing methods. Suitably, the eukaryotic cell is a yeast cell, insect cell, *Drosophila* cell, amphibian cell (e.g. from *Xenopus*), *C. elegans* cell or is a cell of human, non-human primate, equine, bovine, porcine, caprine, ovine, canine, feline, piscine, rodent or murine origin. Non-human animals or cells can be used that are capable of expressing human polypeptides.

[0087] Screening methods also encompass a method of identifying a ligand capable of binding to a peptide biomarker according to the invention, comprising incubating a test substance in the presence of the peptide biomarker in conditions appropriate for binding, and detecting and/or quantifying binding of the peptide to said test substance.

[0088] High-throughput screening technologies based on the biomarkers, uses and methods of the invention, e.g. configured in an array, pattern or signature format, are suitable to monitor biomarker signatures for the identification of potentially useful therapeutic compounds, e.g. ligands such as natural compounds, synthetic chemical compounds (e.g. from combinatorial libraries), peptides, monoclonal or polyclonal antibodies or fragments thereof, capable of binding the biomarker.

[0089] Methods of the invention can be performed in array, pattern or signature format, e.g. on a chip, or as a multiwell array. As described above, other techniques, such as mass spectrometry can also be used. Methods can be adapted into platforms for single tests, or multiple identical or multiple non-identical tests, and can be performed in high throughput format. Methods of the invention may comprise performing one or more additional, different tests to confirm or exclude diagnosis, and/or to further characterise a condition.

[0090] The invention further provides a substance, e.g. a ligand, identified or identifiable by an identification or screening method or use of the invention. Such substances may be capable of stimulating, promoting or activating, directly or indirectly, the activity of a peptide biomarker, or of stimulating, promoting or activating generation of the peptide biomarker. The term substances includes substances that do not directly bind the peptide biomarker and directly induce expression of the peptide biomarker or promote or activate a function, but instead indirectly induce expression of the peptide biomarker or promote/activate a function of the peptide biomarker. Ligands are also included in the term substances; ligands of the invention (e.g. a natural or synthetic chemical compound, peptide, aptamer, oligonucleotide, antibody or antibody fragment) are capable of binding, preferably specific binding, to a peptide biomarker.

[0091] A kit for diagnosing or monitoring a schizophrenic disorder or predisposition thereto may contain one or more components selected from a ligand specific for a peptide biomarker, a peptide biomarker, controls, reagents, and consumables; optionally together with instructions for use of the kit.

[0092] The terms "treating" or "treatment" as used herein with reference to therapeutic uses of the biomarker of the invention describe the management or care of a patient for the purposes of combating disease, and include the administration of the active agents to asymptomatic individuals, for example to prevent the onset of the symptoms or complications (i.e. prophylaxis).

[0093] The term "therapeutic substance" as used herein defines a substance that has therapeutic, i.e. curative/beneficial properties and treats a schizophrenic disorder, alleviates the symptoms thereof or prevents the onset of a schizophrenic disorder. Thus, the substance is for use in the treatment of schizophrenia.

[0094] The following Examples include evidence on which the invention is based.

EXAMPLE 1

[0095] This Example illustrates the utility of miniaturized and parallelized sandwich immunoassays, e.g. Multi-Analyte Profiling tests, highly sensitive assay-systems that allow the accurate quantification of a high number of target proteins in body fluids. These multiplexed assay systems can replace the

traditional single tests and allow the simultaneous determination of a large number of parameters from minute amounts of samples.

[0096] Multi-analyte profiling (maps), using the Luminex XMap® technology platform to measure relative expression levels of 156 biochemical markers, was carried out on serum samples collected from first-onset, drug-naive schizophrenic patients (n=33), prodromal patients (n=17) and well matched healthy controls (n=30). Several biochemical markers were significantly up- or down-regulated in first onset schizophrenic and/or prodromal patients compared to controls and therefore represent candidate biomarkers for use in the early diagnosis of patients potentially suffering from schizophrenia. These markers may also have utility in monitoring patients during treatment or indeed predicting or monitoring drug response, efficacy, side-effects and compliance. The statistically significant candidate biomarkers and the relevant expression data are summarised in Tables 1 to 3. Principal Component Analysis (PCA), using 18 of the most significantly changing biomarkers, clusters schizophrenia samples away from controls with 75% sensitivity and 79% selectivity. Using the same principal components on the prodromal patient samples, they are clustered between the schizophrenics and controls.

TABLE 1

28 analytes changed in schizophrenia patients compared to controls

Analyte	Schizophrenia	Control	p value	Fold change
Beta-2 Microglobulin (µg/ml)	1.13 ± 0.2	1.3 ± 0.4	p = 0.052	−1.15
BDNF (ng/ml)	19.28 ± 5.9	22 ± 5.9	p = 0.072	−1.13
Calcitonin (pg/ml)	3.9 ± 2.3	1.8 ± 0.3	p = 0.007	2.17
IL-13 (pg/ml)	24.69 ± 5.6	35.5 ± 12.5	p = 0.001	−1.35
IL-7 (pg/ml)	44.54 ± 17.2	58.4 ± 23	p = 0.009	−1.31
IL-15 (ng/ml)	0.305 ± 0.11	0.4 ± 0.1	p = 0.009	−1.31
IL-18 (pg/ml)	122.1 ± 52.9	172.6 ± 89.7	p = 0.010	−1.4
IL-3 (ng/ml)	0.032 ± 0.006	0.1 ± 0.023	p = 0.070	−3.13
IL-1ra (pg/ml)	158.7 ± 146.1	90.3 ± 87.9	p = 0.036	1.76
Lymphotactin (ng/ml)	0.159 ± 0.054	0.2 ± 0.1	p = 0.054	1.43
IgM (mg/ml)	0.68 ± 0.3	1.00 ± 0.46	p = 0.004	−1.67
EN-RAGE (ng/ml)	92.3 ± 57.4	58.6 ± 38.2	p = 0.009	1.57
Haptoglobin (mg/ml)	1.25 ± 0.8	0.9 ± 0.5	p = 0.009	1.39
ICAM-1 (ng/ml)	77.9 ± 24.5	93.9 ± 21.9	p = 0.01	
MMP-9 (ng/ml)	25.1 ± 16.7	25.8 ± 9.2	p = 0.012	1.58
Prostatic Acid Phosphatase (ng/ml)	0.318 ± 0.11	0.4 ± 0.1	p = 0.026	−1.25
Stem Cell Factor (pg/ml)	185.96 ± 66	218.3 ± 54.8	p = 0.038	−1.76
Thyroxine Binding Globulin (µg/ml)	57.2 ± 613.6	65.4 ± 32	p = 0.073	−1.15
Tissue Factor (ng/ml)	0.74 ± 0.42	0.6 ± 0.2	p = 0.045	1.23
T3 Antibody	1.79 ± 0.29	1.7 ± 0.33	p = 0.085	1.05
Collage type 4 Antibody	1.627 ± 0.17	1.5 ± 0.17	p = 0.020	1.11
HSP 71	2.8 ± 0.74	2.4 ± 0.63	p = 0.042	1.16

TABLE 1-continued

28 analytes changed in schizophrenia patients compared to controls

Analyte	Schizophrenia	Control	p value	Fold change
Antibody				
Jo-1 Antibody	1.39 ± 0.13	1.3 ± 0.13	p = 0.073	1.06
Mump Antibody	9.6 ± 3.6	7.6 ± 3.6	p = 0.031	1.26
Proteinase 3 (cANCA) Antibody	3.8 ± 0.59	2.5 ± 0.63	p = 0.07	
RNP (c) Antibody	14.7 ± 4.15	12.5 ± 4.9	p = 0.055	1.176
Rubella	322 ± 143	240 ± 147	p = 0.03	1.34
SSB Antibody	2.16 ± 0.23	2.0 ± 0.26	p = 0.027	1.08

TABLE 2

28 analytes changed in prodromal patients compared to controls

Analyte	Schizophrenia	Control	p value	Fold change
Beta-2 Microglobulin (µg/ml)	1.1 ± 0.28	1.3 ± 0.4	p = 0.067	−1.18
BDNF (ng/ml)	17.9 ± 7.1	22 ± 5.9	p = 0.052	−1.23
Calcitonin (pg/ml)	3.8 ± 1.4	1.8 ± 0.3	p = 0.0009	2.11
IL-13 (pg/ml)	26.8 ± 7.7	33.5 ± 12.5	p = 0.028	−1.25
IL-7 (pg/ml)	47.7 ± 16.9	58.4 ± 23	p = 0.074	−1.22
Alpha-1 Antitrypsin (mg/ml)	1.7 ± 0.4	2 ± 0.5	p = 0.058	−1.18
Apolipoprotein A1 (mg/ml)	0.3 ± 0.1	0.4 ± 0.1	p = 0.015	−1.33
CTGF (ng/ml)	5.3 ± 1.6	6.6 ± 2.9	p = 0.0475	−1.25
Fibrinogen (mg/ml)	0.016 ± 0.01	0.030 ± 0.019	p = 0.0023	−1.88
G-CSF (pg/ml)	6.8 ± 2	9.2 ± 3.1	p = 0.032	−1.35
Growth Hormone (ng/ml)	3.9 ± 4.5	1.6 ± 3.1	p = 0.075	2.44
Glutathione S-Transferase (ng/ml)	0.6 ± 0.1	0.8 ± 0.3	p = 0.015	−1.33
IgA (mg/ml)	1.1 ± 0.6	1.7 ± 1.1	p = 0.015	−1.54
IgM (mg/ml)	0.6 ± 0.2	1.0 ± 0.5	p = 0.0006	−1.67
Leptin (ng/ml)	3.1 ± 2.4	8.1 ± 7.9	p = 0.003	−2.63
MDC (pg/ml)	274.4 ± 90.5	349.8 ± 137.9	p = 0.03	−1.28
PAI-1 (ng/ml)	108.3 ± 50.4	145.6 ± 62.3	p = 0.031	−1.35
RANTES (ng/ml)	17.4 ± 6.0	25.2 ± 12.7	p = 0.0062	−1.45
Serum Amyloid P (µg/ml)	18.2 ± 7.8	24.4 ± 11.2	p = 0.033	−1.33
Stem Cell Factor (pg/ml)	192.4 ± 43.6	218.3 ± 54.8	p = 0.083	−1.18
SHBG (nmol/l)	44.0 ± 32.4	93.0 ± 102.6	p = 0.021	−2.13
TNF RII (ng/ml)	2.1 ± 0.6	2.7 ± 0.8	p = 0.007	−1.28
T3 Antibody	1.91 ± 0.37	1.7 ± 0.33	p = 0.023	1.12
Histone H4 Antibody	1.88 ± 0.34	2.1 ± 0.4	p = 0.04	−1.22
HSC 70 Antibody	2.2 ± 0.72	1.9 ± 0.38	p = 0.078	1.16
Rubella	348.0 ± 158.7	240.2 ± 147.6	p = 0.029	1.45
SSB Antibody	2.18 ± 0.21	2.0 ± 0.26	p = 0.021	1.09
V. zoster	24.0 ± 11.7	15.8 ± 7.2	p = 0.015	1.52

TABLE 3

9 analytes significantly changing in both drug naive, first onset schizophrenic and prodromal patients compared to healthy controls.

Analyte	Schizophrenia	Prodromal	Control
Beta-2Microglobulin	1.13 ± 0.2 p = 0.052	1.1 ± 0.3 p = 0.067	1.3 ± 0.4
BDNF	19.28 ± 5.9 p = 0.072	17.9 ± 7.1 p = 0.052	22 ± 5.9
Calcitonin	3.9 ± 2.3 p = 0.007	3.8 ± 1.4 p = 0.001	1.8 ± 0.3
IL-13	24.69 ± 5.6 p = 0.001	26.8 ± 7.7 p = 0.029	35.5 ± 12.5
IL-7	44.54 ± 17.2 p = 0.009	47.7 ± 16.9 p = 0.074	58.4 ± 23
T3-Antibody	1.79 ± 0.286 p = 0.085	1.91 ± 0.368 p = 0.023	1.7 ± 0.33
IgM	0.68 ± 0.3 p = 0.004	0.6 ± 0.22 p = 0.00056	1.00 ± 0.46
Rubella	322 ± 143 p = 0.03	348 ± 158.7 p = 0.029	240 ± 147

[0097] Data analysis of Example 1 indicated that a subset of the components of the acute phase reaction (APR) was significantly altered in both schizophrenia and prodromal patients. Both positive and negative acute phase reactant proteins were significantly changed: haptoglobin increased 1.39 fold between drug naive, first onset schizophrenics and controls ($p \leqq 0.029$), fibrinogen decreased −1.89 fold ($p \leqq 0.02$), ICAM-1 decreased −1.2 fold ($p \leqq 0.010$), α-1 antitrypsin decreased −1.76 fold ($p = 0.058$) and serum Amyloid P decreased −1.43 fold ($p \leqq 0.033$).

EXAMPLE 2

[0098] Further candidate biomarkers have been identified in the same way, with the following results:

TABLE 4

Analyte	p value	FC
Alpha.1.Antitrypsin	0.049989	1.082551
Alpha.2.Macroglobulin	9.75E−05	1.204169
ANG.2 (Angiopoietin.2.)	0.000961	1.321552
Angiotensinogen	0.022697	0.479122
Anti.Nuclear.Antibody	0.000155	1.277376
Apolipoprotein.A1	0.012815	0.855212
Apolipoprotein.CIII	0.031684	0.84917
Apolipoprotein.H	0.023332	1.103593
ASCA (Saccharomyces.cerevisiae Antibody)	0.026127	1.277587
BDNF (.Brain.Derived.Neurotrophic.Factor).	0.009522	0.868904
Beta.2.Microglobulin	6.09E−05	1.186813
Betacellulin	5.14E−05	2.552562
BMP.6	0.001154	2.084057
C..trachomatis	3.67E−06	1.723647
C.Reactive.Protein	0.028894	2.606907
Carcinoembryonic.Antigen	0.00013	1.710968
CD40	0.008796	1.192832
CD40.Ligand	0.002223	0.645895
Centromere.Protein.B.Antibody	2.73E−05	1.420568
CgA.(Chromogranin.A.)	0.007539	1.296562
Cholera.Toxin	0.002769	1.142152
Collagen.Type.2.Antibody	0.002749	1.483716
Collagen.Type.4.Antibody	0.009621	1.089017
Complement.3	0.03407	1.073186
Cortisol	0.021819	1.136634
CTGF (Connective.Tissue.Growth.Factor).	0.006032	1.15326
Cytomegalovirus	0.037969	1.38126
EGF	8.00E−06	0.483268
EGF.R	0.047045	1.105742
ENA.78	0.021286	1.25662
Endothelin.1	0.024661	0.432167
Eotaxin.3	0.002065	2.3159
Epstein.Barr.Virus.Early.Antigen	0.007651	1.554542
Erythropoietin	0.000553	0.509612
Factor.VII	0.015475	0.873992

TABLE 4-continued

Analyte	p value	FC
Fas.Ligand	0.00559	0.575667
Ferritin	0.034533	1.366948
FGF.basic	0.023096	0.684196
Fibrinogen	9.57E−10	0.5385
FSH.(Follicle.Stimulating.Hormone).	0.008361	2.435721
GM.CSF	0.026716	0.528146
GST	7.35E−09	1.305712
H..pylori	0.029218	4.081442
Haptoglobin	6.08E−05	1.655459
Hepatitis.A	0.00024	1.532017
Hepatitis.B.Core	0.01446	1.274912
Hepatitis.B.Surface..Ad.	0.000859	0.571632
Hepatitis.B.Surface..Ay.	0.002927	1.207407
Hepatitis.C.Core	0.000144	1.578016
Hepatitis.C.NS3	0.003394	1.332285
Hepatitis.C.NS4	0.005992	1.511292
Hepatitis.D	0.011377	1.46535
Hepatitis.E.Virus..orf2.6KD.	0.037865	1.225447
Hepatitis.E.Virus..orf3.3KD.	0.0083	0.843731
Herpes.Simplex.Virus.1.2	0.025897	1.40042
HGF..Hepatocyte.growth.factor.	0.016633	1.360862
Histone.Antibody	6.85E−10	1.686243
Histone.H1.Antibody	2.61E−11	2.799757
Histone.H2a.Antibody	0.044772	1.224073
Histone.H2b.Antibody	0.001089	0.788924
Histone.H3.Antibody	1.72E−10	2.415176
HIV.1.gp120	1.66E−06	1.789048
HIV.1.p24	0.038881	1.156733
HSC.70.Antibody	0.00624	1.137019
HSP.32..HO..Antibody	0.004803	0.885912
HSP.71.Antibody	0.00045	1.252665
HSP.90.alpha.Antibody	0.001539	1.791473
HSP.90.beta.Antibody	0.001354	1.623366
ICAM.1	0.009792	1.149373
IgA	0.049771	0.881591
IGF.BP.2	0.018181	1.227532
IL.10	0.000352	1.221097
IL.12p40	0.000201	0.250575
IL.13	0.017859	1.181867
IL.15	0.000165	1.256579
IL.16	0.036951	0.859916
IL.17	5.98E−07	1.677291
IL.18	0.01689	1.22484
IL.1ra	0.005946	1.510124
IL.3	0.008796	0.653955
IL.5	0.00209	0.664767
IL.6	0.012493	43.66667
IL.7	0.019808	1.132941
IL.8	0.005011	1.507679
Influenza.A	0.004233	1.414848
Influenza.A.H3N2	0.000495	1.554768
Insulin	0.025327	1.935424
Insulin.Antibody	0.025375	0.856373

TABLE 4-continued

Analyte	p value	FC
L..donovani	0.001109	0.857349
LH..Luteinizing.Hormone.	0.000997	1.682294
Lyme	0.032584	1.625076
M..pneumoniae	0.046329	1.316456
MDC	0.006304	1.223947
MIF	0.035924	1.725573
MIP.1alpha	0.000169	1.327078
MIP.1beta	0.038342	0.846427
Mitochondrial.Antibody	3.88E-06	1.354705
Mumps	0.001229	1.591415
Myeloperoxidase	0.049584	0.758069
Myeloperoxidase..pANCA..Antibody	0.002673	1.239712
NrCAM	0.029407	0.677036
PAI.1	0.007411	1.182768
Pancreatic.Islet.Cells..GAD..Antibody	0.007054	1.109422
Pancreatic.Polypeptide	0.024602	1.653974
Parainfluenza.1	4.81E-08	2.050935
Parainfluenza.2	0.018508	1.492113
Parainfluenza.3	0.000614	1.674902
PDGF	0.001027	1.379009
Peptide.YY	0.03996	4.420843
PM.1.Antibody	1.81E-05	1.180055
Polio.Virus	2.52E-05	1.284177
Prolactin	0.039821	1.799276
Prostatic.Acid.Phosphatase	0.003273	0.821328
Proteinase.3..cANCA..Antibody	0.000947	1.233907
RANTES	0.039282	1.170133
Resistin	0.008188	0.798165
Respiratory.Syncytial.Virus	0.002998	1.542918
Ribosomal.P.Antibody	0.002013	1.160539
RNP.Antibody	0.000327	1.146123
Rubella	0.043988	1.288998
Rubeola	6.81E-05	1.415315
Scl.70.Antibody	6.94E-05	2.140715
Serum.Amyloid.P	0.00095	1.272045
SGOT	0.000575	1.232216
SHBG	0.045618	0.694279
Smith.Antibody	8.36E-06	1.20085
Sortilin	1.82E-05	0.753436
sRAGE	0.031466	0.751375
SSB.Antibody	4.39E-06	1.193772
Streptolysin.O..SLO.	0.029488	1.367316
T3.Antibody	0.002543	0.849731
TECK	0.014213	1.261096
Thrombopoietin	0.000449	0.838376
Thyroglobulin.Antibody	0.010796	0.847769
TIMP.1	0.00226	1.179578
TNF.alpha	0.040404	1.164689
TNF.RII	0.004678	1.164465
TRAIL.R3	0.031641	0.833379
TSP.1	3.99E-05	0.818264
tTG..Tissue.Transglutaminase..Celiac.-Disease..Antibody	0.002152	1.369371
V..zoster	2.65E-05	1.846248
VEGF	0.003456	0.821183
von.Willebrand.Factor	0.000742	1.51545

[0099] Some or all of the candidate biomarkers identified herein will have potential utility in the diagnosis and monitoring of patients with first onset schizophrenia, acute psychotic episodes or other related psychiatric disorders. These biomarkers are associated with various biochemical pathways: acute phase reaction, oxidative stress, the macrophage response, lipid metabolism; cellular growth and proliferation; and regulation of thyroid hormones. Analysis of these biochemical pathways suggests other potential candidate biomarkers with potential utility in the diagnosis and monitoring of patients with first onset schizophrenia, acute psychotic episodes or other related psychiatric disorders.

[0100] Other immune response system changes were observed in schizophrenics compared to controls. For example, significant decreases in the expression of G-CSF (74% of control; $P<0.034$), ICAM-1 (83%; $P<0.010$), IL-3 (32%; $P<0.074$), IL-7 (76%; $P<0.009$), IL-13 (74%; $P<0.001$) and IL-15 (76%; $P<0.009$) and a robust increase in IL-1 RA (1.76-fold; $P<0.036$) were observed. Such changes indicate decreased white cell production, inflammation (macrophage response and histamine release), B cell proliferation and RA responses in first onset schizophrenics.

[0101] In addition, a separate study using Q-tof MS/MS showed that CD5L decreased significantly (43% $p \leqq 0.002$) in schizophrenia subjects compared to healthy controls. CD5L is able to bind and activate specific cells of the immune system (monocytes and lymphocytes), and plays an important role in the regulation of this system.

[0102] The data also suggest that all or some of these candidate biomarkers (G-CSF, ICAM-1, 3, 7, 13 and 15 with increased expression of IL-1RA) can be used as part of a biomarker assay panel for the early detection of schizophrenia.

[0103] It also follows from this work that first onset schizophrenia patients may show alterations in macrophage, T helper, T Killer and B cell responses. Therefore other potential markers that are already in use for classifying or characterizing these cells will be investigated for their potential application in schizophrenia diagnostics. These include 27E10 and Factor XIII-A for macrophage activation and differentiation, CPM for macrophage maturation, Chitotriosidase and CD14 for macrophage stimulation, as well as CD4 and CD8 for T killer cell differentiation and CD28, CD80 and CD86 for T killer cell activation. Such candidate biomarkers can be used as part of a biomarker assay panel for the early detection of schizophrenia.

1-26. (canceled)

27. A method of diagnosing a psychotic disorder, or predisposition thereto, comprising the steps of:

i. obtaining a first sample from a subject;

ii. measuring the level of peptide biomarkers in the first sample; and

iii. comparing the level of peptide biomarkers in the first sample to a second sample from a control subject that does not have a psychotic disorder,

wherein the peptide biomarkers are Beta-2 Microglobulin, BDNF, Calcitonin, IL-13, IL-7, T3 Antibody, IgM, and Rubella.

28. The method of claim **27**, wherein the peptide biomarkers further comprise IL-15, IL-18, IL-3, IL-1ra, Lymphotactin, EN-RAGE, Haptoglobin, ICAM-1, MMP-9, Prostatic Acid Phosphatase, Stem Cell Factor, Thyroxine Binding Globulin, Tissue Factor, Collage type 4 Antibody, HSP 71 Antibody, Jo-1 Antibody, Mump Antibody, Proteinase 3 (cANCA) Antibody, RNP (c) Antibody, and SSB Antibody.

29. The method according to claim **27**, wherein the level of the peptide biomarkers is detected by analysis of NMR spectra.

30. The method according to claim **27**, wherein the level of the peptide biomarkers is detected by a method selected from NMR, SELDI(-TOF), MALDI(-TOF), 1-D gel-based analysis, 2-D gel-based analysis, mass spectrometry (MS), LC-MS-based techniques, and combinations thereof.

31. The method according to claim **27**, wherein the level of the peptide biomarkers is detected by a method selected from direct or indirect, coupled or uncoupled enzymatic methods, electrochemical, spectrophotometric, fluorimetric, lumino-

metric, spectrometric, polarimetric and chromatographic techniques, or an immunological method such as ELISA.

32. The method according claim **27**, wherein the level of the peptide biomarkers is measured using plasma proteins and is detected by a method selected from the group consisting of ultraviolet absorbance and a colorimetric method.

33. The method according to claim **27**, wherein the level of the peptide biomarkers is detected using a sensor or biosensor comprising one or more enzymes, binding, receptor or transporter proteins, antibody, synthetic receptors or other selective binding molecules for direct or indirect detection of the biomarkers, said detection being coupled to an electrical, optical, acoustic, magnetic or thermal transducer.

34. The method according to claim **27**, wherein the first and second sample is selected from whole blood, blood serum, blood plasma or an extract or purification therefrom, or dilution thereof.

35. The method according to claim **27**, wherein the first and second sample are selected from the group consisting of CSF, urine, saliva, or other bodily fluid, or breath, condensed breath, or an extract or purification therefrom, or dilution thereof.

36. The method according to claim **27**, wherein the subject is drug-naïve.

37. The method according to claim **27**, wherein the subject has a psychotic disorder.

38. The method of claim **37**, wherein the psychotic disorder is schizophrenia.

39. A method of monitoring efficacy of a therapy in a subject having, suspected of having, or of being predisposed to, a psychotic disorder, comprising-the steps of:

i. obtaining a first sample from a subject;

ii. measuring the level of peptide biomarkers in the first sample;

iii. administering therapy to the subject;

iv. obtaining a second sample from a subject;

v. measuring the level of the peptide biomarkers in the second sample; and

vi. comparing the level of peptide biomarkers in the first sample and the second sample,

wherein the peptide biomarkers are Beta-2 Microglobulin, BDNF, Calcitonin, IL-13, IL-7, T3 Antibody, IgM, and Rubella.

40. The method of claim **39**, wherein the peptide biomarkers further comprise IL-15, IL-18, IL-3, IL-1ra, Lymphotactin, EN-RAGE, Haptoglobin, ICAM-1, MMP-9, Prostatic Acid Phosphatase, Stem Cell Factor, Thyroxine Binding Globulin, Tissue Factor, Collage type 4 Antibody, HSP 71 Antibody, Jo-1 Antibody, Mump Antibody, Proteinase 3 (cANCA) Antibody, RNP (c) Antibody, and SSB Antibody.

41. The method according to claim **39**, wherein the first sample taken from the subject is taken prior to commencement of a therapy, and the second sample is taken from the subject at an earlier stage of a therapy.

42. The method according to claim **39**, wherein the therapy is an anti-psychotic disorder therapy.

43. The method according to claim **39**, wherein the level of the peptide biomarkers from the first sample and the level of the peptide biomarkers from the second sample are compared with the level of the peptide biomarkers present in a control.

44. The method according to claim **43**, wherein the control is a normal control that does not have a psychotic disorder

45. The method according to claim **39**, wherein the level of the peptide biomarkers from the first and second sample is detected by analysis of NMR spectra.

46. The method according to claim **39**, wherein the level of the peptide biomarkers from the first and second sample is detected by a method selected from NMR, SELDI(-TOF), MALDI(-TOF), 1-D gel-based analysis, 2-D gel-based analysis, mass spectrometry (MS), LC-MS-based techniques, and combinations thereof.

47. The method according to claim **39**, wherein the level of the peptide biomarkers from the first and second sample is detected by a method selected from direct or indirect, coupled or uncoupled enzymatic methods, electrochemical, spectrophotometric, fluorimetric, luminometric, spectrometric, polarimetric and chromatographic techniques, or an immunological method such as ELISA.

48. The method according claim **39**, wherein the level of the peptide biomarkes from the first and second sample is measured using plasma proteins and is detected by a method selected from the group consisting of ultraviolet absorbance and a colorimetric method.

49. The method according to claim **39**, wherein the level of the peptide biomarkers from the first and second sample is detected using a sensor or biosensor comprising one or more enzymes, binding, receptor or transporter proteins, antibody, synthetic receptors or other selective binding molecules for direct or indirect detection of the biomarkers, said detection being coupled to an electrical, optical, acoustic, magnetic or thermal transducer.

50. The method according to claim **39**, wherein the first and second sample is selected from whole blood, blood serum, blood plasma or an extract or purification therefrom, or dilution thereof.

51. The method according to claim **39**, wherein the first and second sample is selected from the group consisting of CSF, urine, saliva, or other bodily fluid, or breath, condensed breath, or an extract or purification therefrom, or dilution thereof.

52. The method according to claim **39**, wherein the subject is drug-naïve.

53. The method according to claim **39**, wherein the psychotic disorder is a schizophrenic disorder.

54. The method according to claim **53**, wherein the schizophrenic disorder is selected from the group consisting of paranoid, catatonic, disorganized, undifferentiated and residual schizophrenia.

55. A method according claim **39**, wherein the psychotic disorder is a bipolar disorder.

56. A kit for diagnosing a psychotic disorder comprising a biosensor capable of detecting and/or quantifying the peptide biomarkers as defined in claim **27**.

57. The method of claim **56**, wherein the peptide biomarkers further comprise IL-15, IL-18, IL-3, IL-1ra, Lymphotactin, EN-RAGE, Haptoglobin, ICAM-1, MMP-9, Prostatic Acid Phosphatase, Stem Cell Factor, Thyroxine Binding Globulin, Tissue Factor, Collage type 4 Antibody, HSP 71 Antibody, Jo-1 Antibody, Mump Antibody, Proteinase 3 (cANCA) Antibody, RNP (c) Antibody, and SSB Antibody.

58. The method according to claim **56**, wherein the schizophrenic disorder is selected from the group consisting of paranoid, catatonic, disorganized, undifferentiated and residual schizophrenia.

59. A kit for monitoring a psychotic disorder comprising a biosensor capable of detecting and/or quantifying the peptide biomarkers as defined in claim **27**.

60. The method of claim **59**, wherein the peptide biomarkers further comprise IL-15, IL-18, IL-3, IL-1ra, Lymphotactin, EN-RAGE, Haptoglobin, ICAM-1, MMP-9, Prostatic Acid Phosphatase, Stem Cell Factor, Thyroxine Binding Globulin, Tissue Factor, Collage type 4 Antibody, HSP 71 Antibody, Jo-1 Antibody, Mump Antibody, Proteinase 3 (cANCA) Antibody, RNP (c) Antibody, and SSB Antibody.

61. The method according to claim **59**, wherein the schizophrenic disorder is selected from the group consisting of paranoid, catatonic, disorganized, undifferentiated and residual schizophrenia.

62. A diagnostic test for the diagnosis, predisposition, or monitoring of schizophrenia, comprising quantifying peptide biomarkers, wherein the peptide biomarkers are Beta-2 Microglobulin, BDNF, Calcitonin, IL-13, IL-7, T3 Antibody, IgM, and Rubella.

63. The method of claim **62**, wherein the peptide biomarkers further comprise IL-15, IL-18, IL-3, IL-1ra, Lymphotactin, EN-RAGE, Haptoglobin, ICAM-1, MMP-9, Prostatic Acid Phosphatase, Stem Cell Factor, Thyroxine Binding Globulin, Tissue Factor, Collage type 4 Antibody, HSP 71 Antibody, Jo-1 Antibody, Mump Antibody, Proteinase 3 (cANCA) Antibody, RNP (c) Antibody, and SSB Antibody.

* * * * *

专利名称(译)	用于诊断和监测精神病的方法和生物标志物		
公开(公告)号	US20110165554A1	公开(公告)日	2011-07-07
申请号	US12/809269	申请日	2008-12-18
[标]申请(专利权)人(译)	剑桥企业有限公司		
申请(专利权)人(译)	PSYNOVA NEUROTECH有限公司 剑桥企业有限公司		
[标]发明人	LEVIN YISHAI BAHN SABINE WANG LAN SCHWARZ EMANUEL MCALLISTER GEORGE		
发明人	LEVIN, YISHAI BAHN, SABINE WANG, LAN SCHWARZ, EMANUEL MCALLISTER, GEORGE		
IPC分类号	C12Q1/70 C12Q1/42 C12Q1/37 C12Q1/25 G01N33/53 C12Q1/02 C12N9/00 G01R33/46 G01N30/02 G01N21/33 G01J3/46 G01N33/48 H01J49/26		
CPC分类号	G01N33/6893 G01N2800/30 G01N2800/50 G01N33/6896 G01R33/465 G01N2800/302 G01N2800/52		
优先权	2007024735 2007-12-19 GB		
外部链接	Espacenet USPTO		

摘要(译)

诊断或监测精神病或其倾向的方法包括在取自受试者的样品中测量本文列出的一种或多种肽生物标志物的水平。

TABLE 3

9 analytes significantly changing in both drug naive, first onset schizophrenic and prodromal patients compared to healthy controls.

Analyte	Schizophrenia	Prodromal	Control
Beta-2Microglobulin	1.13 ± 0.2 p = 0.052	1.1 ± 0.3 p = 0.067	1.3 ± 0.4
BDNF	19.28 ± 5.9 p = 0.072	17.9 ± 7.1 p = 0.052	22 ± 5.9
Calcitonin	3.9 ± 2.3 p = 0.007	3.8 ± 1.4 p = 0.001	1.8 ± 0.3
IL-13	24.69 ± 5.6 p = 0.001	26.8 ± 7.7 p = 0.029	35.5 ± 12.5
IL-7	44.54 ± 17.2 p = 0.009	47.7 ± 16.9 p = 0.074	58.4 ± 23
T3-Antibody	1.79 ± 0.286 p = 0.085	1.91 ± 0.368 p = 0.023	1.7 ± 0.33
IgM	0.68 ± 0.3 p = 0.004	0.6 ± 0.22 p = 0.00056	1.00 ± 0.46
Rubella	322 ± 143 p = 0.03	348 ± 158.7 p = 0.029	240 ± 147