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Fortsættes ...

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Description

BACKGROUND OF THE INVENTION

[0001] Clinical depression, including both bipolar disorders and major depression disorders, is a major public health problem, affecting an estimated 9.5% of the adult population of the United States each year. While it has been hypothesized that mental illness, including mood disorders such as major depression ("MDD") and bipolar disorder ("BP") as well as psychotic disorders such as schizophrenia, may have genetic roots, little progress has been made in identifying gene sequences and gene products that play a role in causing these disorders, as is true for many diseases with a complex genetic origin (*see, e.g.*, Burmeister, Biol. Psychiatry 45:522-532 (1999)).

[0002] The current lack of biomarkers and the ineffectiveness and reliability of the diagnosis and rates are important issues for the treatment of mental disorders. For example, around 15% of the population suffers from MDD while approximately 1% suffers from BP disorders. Diagnosing bipolar disorder is difficult when, as sometimes occurs, the patient presents only symptoms of depression to the clinician. At least 10-15% ofBP patients are reported to be misdiagnosed as MDD. The consequences of such misdiagnosis include a delay in being introduced to efficacious treatment with mood stabilizers and a delay in seeking or obtaining counseling specific to bipolar disorder. Also treatment with antidepressants alone induces rapid cycling, switching to manic or mixed state, and consequently increases the risk of suicide. Furthermore, in addition to a lack of efficacy, long onset of action and side effects (sexual, sleep, weight gain, *etc.),* there are recent concerns relating to the undesirable effects of antidepressants on metabolic syndromes, such as diabetes and hypercholesteremia.

[0003] Clearly, there is a need for methods of obtaining accurate and objective information about the physiological and/or genetic status of depressed or potentially suicidal patients, particularly as the patient's physiological and/or genetic

status relates to the likely response of the patient to a particular treatment regimen.

[0004] WO 2004/056865 discloses compounds capable of modulating the FGFR ligand interaction. Further, WO 2004/056865 discloses an NCAM binding motif peptide.

[0005] WO 2005/014623 also discloses a NCAM FGFR binding motif peptide.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides a ligand that activates FGF receptor for use as an antidepressant in the therapeutic treatment of an individual with major depressive disorder (MDD), wherein the ligand is an NCAM peptide mimetic. Both FGF2 and the NCAM peptide mimetic have antidepressant-like effects in the forced swim test when injected intracerebroventricularly. The description and examples presented herein show that the presence of a peptide inhibitor reverses the effect.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007]

FIGURE 1 shows FGFR2 variant differences in Mood Disorders. FGFR2 soluble receptor splice variants may represent a smaller percentage of the total receptors in MDD than in controls.

FIGURE 2 shows the effect of acute injection of FGF2 on mouse depression and anxiety, as measured by mobility tests (top) and the elevated plus maze (EPM) test (bottom), respectively. "Open," "center," and "closed" refer to time spent in the open, center and closed parts of the EPM, respectively.

FIGURE 3 shows the effects of injections of NCAM peptide on mouse depression and anxiety, as measured by the climbing and forced swim test (top) and

the elevated plus maze (EPM) test (bottom), respectively. "Open," "center," and "closed" refer to time spent in the open, center and closed parts of the EPM, respetively.

FIGURE 4 shows the effects of injections of a peptide inhibitor on mouse depression and anxiety, as measured by the climbing and forced swim test (top) and the elevated plus maze (EPM) test (bottom), respectively. "Open," "center," and "closed" refer to time spent in the open, center and closed parts of the EPM, respetively.

FIGURE 5 shows a table listing genes in the cAMP signalling pathway whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) from patients with bipolor disorder (BPD).

FIGURE 6 shows a table listing genes in cAMP signalling pathways whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) of patients with major depression disorder (MDD).

FIGURE 7 shows a table listing genes in the phosphatidylinositol signalling pathway whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) of patients with bipolar disorder (BPD).

FIGURE 8 shows a table listing genes in the phosphatidylinositol signalling pathway whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) of patients with major depression disorder (MDD).

FIGURE 9 shows two charts which illustrate the effect of chronic FGF-2 administration (5ng/g, 3 weeks) on anxiety in rodents with different, as measured by the time the rodents spend in the light compartment of the test system. LR, animals with intrinsic high anxiety; HR, animals with intrinsic low anxiety; HRFGF-2, low anxiety animals administered FGF-2; LRFGF-2, high anxiety animals administered FGF-2.

FIGURE 10 shows the inverse relationship between FGF-2 gene expression and anxiety behavior using the "open arms" test. CA-2, hippocampus region CA-2.

FIGURE 11, top, shows a schematic of the basic structure of FGFR2 and FGFR3 aligned with the exons amplified and described in the Example. Emphasis is placed on the IIIb/IIIc splice variants in the C-terminus of the third Ig-like domain of both receptors (R2 and R3). Exon sequences for FGFR2 and FGFR3 are in no way identical (see FIGURE 11, bottom), but exon nomenclature was synchronized to match each exon number to corresponding regions on both R2 and R3 protein structures. The truncated and cleaved isoforms of the FGF receptors are excluded from the schematic. FIGURE 11, bottom, shows sequences of forward and reverse FGFR2 and FGFR3 primers designed for real time RT-PCR quantitative analysis. Primers were optimized and designed for maximum efficiency with differential detection for IIIb/IIIc splice variants for both FGFR2 and FGFR3.

FIGURE 12 shows two charts which illustrate the chronic stress-induced decrease in the exon IIIc:IIIb splice variant expression ratio in both FGFR2 (top panel) and FGFR3 (bottom panel). V (vehicle); NS (non-stress); Chronic stress (S); FGF-2 (F).

DEFINITIONS

[0008] A "mental disorder" or "mental illness" or "mental disease" or "psychiatric or neuropsychiatric disease or illness or disorder" refers to mood disorders (*e.g.*, major depression, mania, and bipolar disorders), psychotic disorders (*e.g.*, schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic disorder, and shared psychotic disorder), personality disorders, anxiety disorders (*e.g.*, obsessive-compulsive disorder) as well as other mental disorders such as substance -related disorders, childhood disorders, dementia, autistic disorder, adjustment disorder, delirium, multi-infarct dementia, and Tourette's disorder as described in Diagnostic and Statistical

Manual of Mental Disorders, Fourth Edition, (DSM IV). Typically, such disorders have a complex genetic and/or a biochemical component.

[0009] A "mood disorder" refers to disruption of feeling tone or emotional state experienced by an individual for an extensive period of time. Mood disorders include major depression disorder (*i.e.*, unipolar disorder), mania, dysphoria, bipolar disorder, dysthymia, cyclothymia and many others. *See, e.g.*, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, (DSM IV).

[0010] "Major depression disorder," "major depressive disorder," or "unipolar disorder" refers to a mood disorder involving any of the following symptoms: persistent sad, anxious, or "empty" mood; feelings of hopelessness or pessimism; feelings of guilt, worthlessness, or helplessness; loss of interest or pleasure in hobbies and activities that were once enjoyed, including sex; decreased energy, fatigue, being "slowed down"; difficulty concentrating, remembering, or making decisions; insomnia, early-morning awakening, or oversleeping; appetite and/or weight loss or overeating and weight gain; thoughts of death or suicide or suicide attempts; restlessness or irritability; or persistent physical symptoms that do not respond to treatment, such as headaches, digestive disorders, and chronic pain. Various subtypes of depression are described in, *e.g.*, DSM IV.

[0011] "Bipolar disorder" is a mood disorder characterized by alternating periods of extreme moods. A person with bipolar disorder experiences cycling of moods that usually swing from being overly elated or irritable (mania) to sad and hopeless (depression) and then back again, with periods of normal mood in between. Diagnosis of bipolar disorder is described in, *e.g.*, DSM IV. Bipolar disorders include bipolar disorder I (mania with or without major depression) and bipolar disorder II (hypomania with major depression), see, *e.g.*, DSM IV.

[0012] "A psychotic disorder" refers to a condition that affects the mind, resulting in at least some loss of contact with reality. Symptoms of a psychotic disorder include, *e.g.*, hallucinations, changed behavior that is not based on reality,

delusions and the like. *See, e.g.*, DSM IV. Schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic disorder, substance-induced psychotic disorder, and shared psychotic disorder are examples of psychotic disorders.

[0013] "Schizophrenia" refers to a psychotic disorder involving a withdrawal from reality by an individual. Symptoms comprise for at least a part of a month two or more of the following symptoms: delusions (only one symptom is required if a delusion is bizarre, such as being abducted in a space ship from the sun); hallucinations (only one symptom is required if hallucinations are of at least two voices talking to one another or of a voice that keeps up a running commentary on the patient's thoughts or actions); disorganized speech (*e.g.*, frequent derailment or incoherence); grossly disorganized or catatonic behavior; or negative symptoms, *i.e.*, affective flattening, alogia, or avolition. Schizophrenia encompasses disorders such as, *e.g.*, schizoaffective disorders. Diagnosis of schizophrenia is described in, *e.g.*, DSM IV. Types of schizophrenia include, *e.g.*, paranoid, disorganized, catatonic, undifferentiated, and residual.

[0014] An "antidepressant" refers to an agents typically used to treat clinical depression. Antidepressants includes compounds of different classes including, for example, specific serotonin reuptake inhibitors (*e.g.*, fluoxetine), tricyclic antidepressants (*e.g.*, desipramine), and dopamine reuptake inhibitors (e.g, bupropion). Typically, antidepressants of different classes exert their therapeutic effects via different biochemical pathways. Often these biochemical pathways overlap or intersect. Additional diseases or disorders often treated with antidepressants include, chronic pain, anxiety disorders, and hot flashes.

[0015] An "agonist" refers to an agent that binds to a polypeptide or polynucleotide, stimulates, increases, activates, facilitates, enhances activation, sensitizes or up regulates the activity or expression of a polypeptide or polynucleotide.

[0016] An "antagonist" refers to an agent that inhibits expression of a polypeptide or polynucleotide or binds to, partially or totally blocks stimulation, decrea-

ses, prevents, delays activation, inactivates, desensitizes, or down regulates the activity of a polypeptide or polynucleotide.

[0017] "Inhibitors," "activators," and "modulators" of expression or of activity are used to refer to inhibitory, activating, or modulating molecules, respectively, identified using *in vitro* and *in vivo* assays for expression or activity, *e.g.*, ligands, agonists, antagonists, and their homologs and mimetics. The term "modulator" includes inhibitors and activators. Inhibitors are agents that, *e.g.*, inhibit expression of a polypeptide or polynucleotide or bind to, partially or totally block stimulation or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide, *e.g.*, antagonists. Activators are agents that, *e.g.*, induce or activate the expression of a polypeptide or polynucleotide or bind to, stimulate, increase, open, activate, facilitate, enhance activation or enzymatic activity, sensitize or up regulate the activity of a polypeptide or polynucleotide, *e.g.*, agonists. Modulators include naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Assays to identify inhibitors and activators include, *e.g.*, applying putative modulator compounds to cells, in the presence or absence of a polypeptide or polynucleotide and then determining the functional effects on a polypeptide or polynucleotide activity. Samples or assays comprising a polypeptide or polynucleotide that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative activity value of 100%. Inhibition is achieved when the activity value of a polypeptide or polynucleotide relative to the control is about 80%, optionally 50% or 25-1%. Activation is achieved when the activity value of a polypeptide or polynucleotide relative to the control is 110%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

[0018] The term "test compound" or "drug candidate" or "modulator" or grammatical equivalents as used herein describes any molecule, either naturally occurring or synthetic, *e.g.*, protein, oligopeptide (*e.g.*, from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in

length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, lipid, fatty acid, polynucleotide, RNAi, oligonucleotide, etc. The test compound can be in the form of a library of test compounds, such as a combinatorial or randomized library that provides a sufficient range of diversity. Test compounds are optionally linked to a fusion partner, *e.g.*, targeting compounds, rescue compounds, dimerization compounds, stabilizing compounds, addressable compounds, and other functional moieties. Conventionally, new chemical entities with useful properties are generated by identifying a test compound (called a "lead compound") with some desirable property or activity, *e.g.*, inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput screening (HTS) methods are employed for such an analysis.

[0019] The term "Table #" when used herein includes all sub-tables of the Table referred to (*e.g.*, "Table 1" refers to Table 1A, 1B, and Table 1C) unless otherwise indicated.

[0020] "Determining the functional effect" refers to assaying for a compound that increases or decreases a parameter that is indirectly or directly under the influence of a polynucleotide or polypeptide (such as a polynucleotide of FIGURE 1, FIGURES 5-8, or Tables 1-4, or a polypeptide encoded by a gene of FIGURE 1, FIGURES 5-8, or Tables 1-4), *e.g.*, measuring physical and chemical or phenotypic effects. Such functional effects can be measured by any means known to those skilled in the art, *e.g.*, changes in spectroscopic (*e.g.*, fluorescence, absorbance, refractive index), hydrodynamic (*e.g.*, shape), chromatographic, or solubility properties for the protein; measuring inducible markers or transcriptional activation of the protein; measuring binding activity or binding assays, *e.g.* binding to antibodies; measuring changes in ligand binding affinity; measurement of calcium influx; measurement of the accumulation of an enzymatic product of a polypeptide or depletion of an substrate; measurement of changes in protein levels of a polypeptide; measurement of RNA stability; G-protein binding; GPCR phosphorylation or dephosphorylation; signal transduction, *e.g.*, receptor-ligand interactions, second messenger concentrations (*e.g.*,

cAMP, IP3, or intracellular Ca^{2+}); identification of downstream or reporter gene expression (CAT, luciferase, β-gal, GFP and the like), *e.g.*, via chemiluminescence, fluorescence, colorimetric reactions, antibody binding, inducible markers, and ligand binding assays.

[0021] Samples or assays comprising a nucleic acid or protein disclosed herein that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of inhibition. Control samples (untreated with inhibitors) are assigned a relative protein activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 80%, preferably 50%, more preferably 25-0%. Activation is achieved when the activity value relative to the control (untreated with activators) is 110%, more preferably 150%, more preferably 200-500% (*i.e.*, two to five fold higher relative to the control), more preferably 1000-3000% higher.

[0022] "Biological sample" includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histologic purposes. Such samples include blood, sputum, tissue, lysed cells, brain biopsy, cultured cells, *e.g.*, primary cultures, explants, and transformed cells, stool, urine, *etc.* A biological sample is typically obtained from a eukaryotic organism, most preferably a mammal such as a primate, *e.g.*, chimpanzee or human; cow; dog; cat; a rodent, *e.g.*, guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

[0023] The terms "peptidomimetic" and "mimetic" refer to a synthetic chemical compound that has substantially the same structural and functional characteristics of the polynucleotides, polypeptides, antagonists or agonists disclosed herein. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics" (Fauchere, Adv. Drug Res. 15:29 (1986); Veber and Freidinger TINS p. 392 (1985); and Evans et al., J. Med. Chem. 30:1229 (1987). Peptide mimetics that are structurally similar to therapeutically useful peptides may be

used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biological or pharmacological activity), such as a CCX CKR, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of, *e.g.*, - CH_2NH-, -CH_2S-, -CH_2-CH_2-, -CH=CH- (cis and trans), -$COCH_2$-, -$CH(OH)CH_2$-, and - CH_2SO-. The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. For example, a mimetic composition is capable of carrying out the binding or enzymatic activities of a polypeptide or polynucleotide or inhibiting or increasing the enzymatic activity or expression of a polypeptide or polynucleotide.

[0024] The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

[0025] The term "isolated," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It is preferably in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated gene is separated from open reading frames that flank the gene and encode a protein other than the gene of interest. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

[0026] The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Cassol *et al.* (1992); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[0027] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent peptide bonds.

[0028] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound

to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0029] Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0030] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids that encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0031] As to amino acid sequences, one of skill will recognize that individual

substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles.

[0032] The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

(*see, e.g.*, Creighton, Proteins (1984)).

[0033] "Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0034] The terms "identical" or percent "identity," in the context of two or more

nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length.

[0035] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0036] A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA

in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g.*, Ausubel et al., Current Protocols in Molecular Biology (1995 supplement)).

[0037] An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) Nuc. Acids Res. 25:3389-3402, and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix *(see* Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0038] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0039] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0040] The phrase "a nucleic acid sequence encoding" refers to a nucleic acid that contains sequence information for a structural RNA such as rRNA, a tRNA, or the primary amino acid sequence of a specific protein or peptide, or a binding site for a trans-acting regulatory agent. This phrase specifically encompasses degenerate codons (*i.e.*, different codons which encode a single amino acid) of the native sequence or sequences which may be introduced to conform with codon preference in a specific host cell.

[0041] The term "recombinant" when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or pro-

tein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (nonrecombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all.

[0042] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (*e.g.*, a fusion protein).

[0043] An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter.

[0044] One who is "predisposed for a mental disorder" as used herein means a person who has an inclination or a higher likelihood of developing a mental disorder when compared to an average person in the general population.

DETAILED DESCRIPTION OF THE INVENTION

[0045] Evidence based on analysis of only a restricted number of molecules suggests altered and unique gene disregulation that may be involved in the pathophysiology of bipolar disorder (BPD) and major depressive disorder (MDD) as well as in the mechanism of drug treatment for these disorders. The

recent development of microarray technology allows a comprehensive view of the mRNA expression profiles of specific genes, systems and signaling pathways.

I. Introduction

[0046] To understand the genetic basis of mental disorders, studies have been conducted to investigate the expression patterns of genes that are differentially expressed specifically in central nervous system of subjects with mood disorders. In several studies, the differential and unique expression of known and novel genes was determined by way of interrogating total RNA samples purified from postmortem brains of BP and MDD patients with Affymetrix Gene Chips® (containing high-density oligonucleotide probe set arrays). The fundamental principle is that by identifying genes and pathways that are differentially expressed in BP and/or MDD (relative to healthy control subjects), via global expression profiling of the transcriptomes as above, one can identify genes that cause, effect, or are associated with the disease, or that interact with drugs used to treat the disease, for use in diagnostic and therapeutic applications.

[0047] The Examples provided herein describe the microarray gene expression profiling of the dorsolateral prefrontal, anterior cingulate, hippocampus, Nucleus Accumbens, Amigdala and cerebellar cortices of BPD and MDD patients. In particular, the mRNA expression levels of genes related to the FGF and GPCRs pathways are disclosed. The detection of splice variants of FGFR2 is also disclosed, which are also dysregulated. Genes which are disregulated by lithium for the specific treatment of BP disorders are also provided.

[0048] Methods for exploiting the altered expression (either higher or lower expression as indicated herein) or unique differential expression of the genes of FIGURE 1, FIGURES 5-8, or Tables 1-4 which is observed in selected brain regions of patients diagnosed with mood disorders (*e.g.*, bipolar disorder and major depression disorder) in comparison with normal individuals are provided.

[0049] Methods of identifying a compound useful for the treatment of such disorders by selecting compounds, *e.g.*, FGF2, NCAM and peptide inhibitors of the FGF system, that modulate the functional effect of the translation products or the expression of the transcripts described herein are disclosed. The invention also provides compounds for use in methods of treating patients with such mental disorders.

[0050] Antidepressants belong to different classes, *e.g.*, desipramine, bupropion, and fluoxetine are in general equally effective for the treatment of clinical depression, but act by different mechanisms. The similar effectiveness of the drugs for treatment of mood disorders suggests that they act through a presently unidentified common pathway. Animal models of depression, including treatment of animals with known therapeutics such as SSRIs, can be used to examine the mode of action of the genes provided herein. Lithium is drug of choice for treating BP.

II. General Recombinant nucleic acid methods

[0051] Polynucleotides may be isolated and cloned using recombinant methods. Such polynucleotides include, *e.g.*, those listed in FIGURE 1, FIGURES 5-8, or Tables 1-4, which can be used for, *e.g.*, protein expression or during the generation of variants, derivatives, expression cassettes, to monitor gene expression, for the isolation or detection of sequences in different species, for diagnostic purposes in a patient, *e.g.*, to detect mutations or to detect expression levels of nucleic acids or polypeptides. The sequences may be operably linked to a heterologous promoter. The nucleic acids may be are from any mammal, including, in particular, *e.g.*, a human, a mouse, a rat, a primate, *etc.*

A. General Recombinant Nucleic Acids Methods

[0052] Routine techniques in the field of recombinant genetics are relied on. Basic texts disclosing the general methods include Sambrook et al., Molecular Cloning, A Laboratory Manual (3rd ed. 2001); Kriegler, Gene Transfer and Ex-

pression: A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel et al., eds., 1994)).

[0053] For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Proteins sizes are estimated from gel electrophoresis, from sequenced proteins, from derived amino acid sequences, or from published protein sequences.

[0054] Oligonucleotides that are not commercially available can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, Tetrahedron Letts. 22:1859-1862 (1981), using an automated synthesizer, as described in Van Devanter et. al., Nucleic Acids Res. 12:6159-6168 (1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, J. Chrom. 255:137-149 (1983).

[0055] The sequence of the cloned genes and synthetic oligonucleotides can be verified after cloning using, *e.g.*, the chain termination method for sequencing double-stranded templates of Wallace et al., Gene 16:21-26 (1981).

B. Cloning Methods for the Isolation of Nucleotide Sequences Encoding Desired Proteins

[0056] In general, the nucleic acids encoding the subject proteins are cloned from DNA sequence libraries that are made to encode cDNA or genomic DNA. The particular sequences can be located by hybridizing with an oligonucleotide probe, the sequence of which can be derived from the sequences of the genes listed in FIGURE 1, FIGURES 5-8, or Tables 1-4, which provide a reference for PCR primers and defines suitable regions for isolating specific probes. Alternatively, where the sequence is cloned into an expression library, the expressed recombinant protein can be detected immunologically with antisera or purified

antibodies made against a polypeptide comprising an amino acid sequence encoded by a gene listed in FIGURE 1, FIGURES 5-8, or Tables 1-4.

[0057] Methods for making and screening genomic and cDNA libraries are well known to those of skill in the art (*see, e.g.*, Gubler and Hoffman Gene 25:263-269 (1983); Benton and Davis Science, 196:180-182 (1977); and Sambrook, *supra*). Brain cells are an example of suitable cells to isolate RNA and cDNA sequences.

[0058] Briefly, to make the cDNA library, one should choose a source that is rich in mRNA. The mRNA can then be made into cDNA, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. For a genomic library, the DNA is extracted from a suitable tissue and either mechanically sheared or enzymatically digested to yield fragments of preferably about 5-100 kb. The fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged *in vitro,* and the recombinant phages are analyzed by plaque hybridization. Colony hybridization is carried out as generally described in Grunstein et al., Proc. Natl. Acad. Sci. USA., 72:3961-3965 (1975).

[0059] An alternative method combines the use of synthetic oligonucleotide primers with polymerase extension on an mRNA or DNA template. Suitable primers can be designed from specific sequences. This polymerase chain reaction (PCR) method amplifies the nucleic acids encoding the protein of interest directly from mRNA, cDNA, genomic libraries or cDNA libraries. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also be useful, for example, to clone nucleic acids encoding specific proteins and express said proteins, to synthesize nucleic acids that will be used as probes for detecting the presence of mRNA encoding a polypeptide in physiological samples, for nucleic acid sequencing, or for other purposes *(see,* U.S. Patent Nos. 4,683,195 and

4,683,202). Genes amplified by a PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

[0060] Appropriate primers and probes for identifying polynucleotides from mammalian tissues can be derived from the sequences provided herein. For a general overview of PCR, *see*, Innis *et al. PCR Protocols: A Guide to Methods and Applications, Academic Press,* San Diego (1990).

[0061] Synthetic oligonucleotides can be used to construct genes. This is done using a series of overlapping oligonucleotides, usually 40-120 bp in length, representing both the sense and anti-sense strands of the gene. These DNA fragments are then annealed, ligated and cloned.

[0062] A gene encoding a polypeptide can be cloned using intermediate vectors before transformation into mammalian cells for expression. These intermediate vectors are typically prokaryote vectors or shuttle vectors. The proteins can be expressed in either prokaryotes, using standard methods well known to those of skill in the art, or eukaryotes as described *infra*.

III. Purification of proteins

[0063] Either naturally occurring or recombinant polypeptides can be purified for use in functional assays. Naturally occurring polypeptides, *e.g.*, polypeptides encoded by genes listed in FIGURE 1, FIGURES 5-8, or Tables 1-4, can be purified, for example, from mouse or human tissue such as brain or any other source of an ortholog. Recombinant polypeptides can be purified from any suitable expression system.

[0064] The polypeptides may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (*see, e.g.*, Scopes, Protein Purification: Principles and Practice (1982); U.S. Patent No. 4,673,641; Ausubel *et al., supra;* and Sambrook *et al., supra*).

[0065] A number of procedures can be employed when recombinant polypeptides are purified. For example, proteins having established molecular adhesion properties can be reversible fused to polypeptides. With the appropriate ligand, the polypeptides can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. Finally the polypeptide can be purified using immunoaffinity columns.

A. Purification of Proteins from Recombinant Bacteria

[0066] When recombinant proteins are expressed by the transformed bacteria in large amounts, typically after promoter induction, although expression can be constitutive, the proteins may form insoluble aggregates. There are several protocols that are suitable for purification of protein inclusion bodies. For example, purification of aggregate proteins (hereinafter referred to as inclusion bodies) typically involves the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells typically, but not limited to, by incubation in a buffer of about 100-150 µg/ml lysozyme and 0.1% Nonidet P40, a non-ionic detergent. The cell suspension can be ground using a Polytron grinder (Brinkman Instruments, Westbury, NY). Alternatively, the cells can be sonicated on ice. Alternate methods of lysing bacteria are described in Ausubel *et al.* and Sambrook *et al.*, both *supra*, and will be apparent to those of skill in the art.

[0067] The cell suspension is generally centrifuged and the pellet containing the inclusion bodies resuspended in buffer which does not dissolve but washes the inclusion bodies, *e.g.*, 20 mM Tris-HCl (pH 7.2), 1 mM EDTA, 150 mM NaCl and 2% Triton-X 100, a non-ionic detergent. It may be necessary to repeat the wash step to remove as much cellular debris as possible. The remaining pellet of inclusion bodies may be resuspended in an appropriate buffer (*e.g.*, 20 mM sodium phosphate, pH 6.8, 150 mM NaCl). Other appropriate buffers will be apparent to those of skill in the art.

[0068] Following the washing step, the inclusion bodies are solubilized by the

addition of a solvent that is both a strong hydrogen acceptor and a strong hydrogen donor (or a combination of solvents each having one of these properties). The proteins that formed the inclusion bodies may then be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to, urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents that are capable of solubilizing aggregate-forming proteins, such as SDS (sodium dodecyl sulfate) and 70% formic acid, are inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of the immunologically and/or biologically active protein of interest. After solubilization, the protein can be separated from other bacterial proteins by standard separation techniques.

[0069] Alternatively, it is possible to purify proteins from bacteria periplasm. Where the protein is exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to those of skill in the art (*see*, Ausubel *et al., supra*). To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is resuspended in ice-cold 5 mM $MgSO_4$ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be separated from the host proteins by standard separation techniques well known to those of skill in the art.

B. Standard Protein Separation Techniques For Purifying Proteins

1. <u>Solubility Fractionation</u>

[0070] Often as an initial step, and if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol is to add saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This will precipitate the most hydrophobic proteins. The precipitate is discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, through either dialysis or diafiltration. Other methods that rely on solubility ofproteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

2. <u>Size Differential Filtration</u>

[0071] Based on a calculated molecular weight, a protein of greater and lesser size can be isolated using ultrafiltration through membranes of different pore sizes (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

3. <u>Column Chromatography</u>

[0072] The proteins of interest can also be separated from other proteins on the basis of their size, net surface charge, hydrophobicity and affinity for ligands. In

addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art.

[0073] It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (*e.g.*, Pharmacia Biotech).

IV. Screening for modulators of polypeptides and polynucleotides

[0074] Modulators of polypeptides or polynucleotides, *i.e.* agonists or antagonists of their activity or modulators of polypeptide or polynucleotide expression, are useful for treating a number of human diseases, including mood disorders or psychotic disorders. Administration of agonists, antagonists or other agents that modulate expression of the polynucleotides or polypeptides can be used to treat patients with mood disorders or psychotic disorders.

A. Screening methods

[0075] A number of different screening protocols can be utilized to identify agents that modulate the level of expression or activity ofpolypeptides and polynucleotides in cells, particularly mammalian cells, and especially human cells. In general terms, the screening methods involve screening a plurality of agents to identify an agent that modulates the polypeptide activity by binding to a polypeptide, modulating inhibitor binding to the polypeptide or activating expression of the polypeptide or polynucleotide, for example.

1. <u>Binding Assays</u>

[0076] Preliminary screens can be conducted by screening for agents capable of binding to a polypeptide, as at least some of the agents so identified are likely modulators of polypeptide activity. The binding assays usually involve contacting a polypeptide with one or more test agents and allowing sufficient time for

the protein and test agents to form a binding complex. Any binding complexes formed can be detected using any of a number of established analytical techniques. Protein binding assays include, but are not limited to, methods that measure co-precipitation, co-migration on non-denaturing SDS-polyacrylamide gels, and co-migration on Western blots (*see, e.g.*, Bennet and Yamamura, (1985) "Neurotransmitter, Hormone or Drug Receptor Binding Methods," in Neurotransmitter Receptor Binding (Yamamura, H. I., et al., eds.), pp. 61-89. The protein utilized in such assays can be naturally expressed, cloned or synthesized.

2. Catalytic activity

[0077] Catalytic activity of polypeptides can be determined by measuring the production of enzymatic products or by measuring the consumption of substrates. Activity refers to either the rate of catalysis or the ability to the polypeptide to bind (K_m) the substrate or release the catalytic product (K_d).

[0078] Analysis of the activity of polypeptides are performed according to general biochemical analyses. Such assays include cell-based assays as well as *in vitro* assays involving purified or partially purified polypeptides or crude cell lysates. The assays generally involve providing a known quantity of substrate and quantifying product as a function of time.

3. Validation

[0079] Agents that are initially identified by any of the foregoing screening methods can be further tested to validate the apparent activity. Preferably such studies are conducted with suitable animal models. The basic format of such methods involves administering a lead compound identified during an initial screen to an animal that serves as a model for humans and then determining if expression or activity of a polynucleotide or polypeptide is in fact upregulated. The animal models utilized in validation studies generally are mammals of any kind. Specific examples of suitable animals include, but are not limited to, pri-

mates, mice, and rats. As described herein, models using admininstration of known therapeutics can be useful.

4. Animal models

[0080] Animal models of mental disorders also find use in screening for modulators. Invertebrate models such as *Drosophila* models can be used, screening for modulators of *Drosophila* orthologs of the human genes disclosed herein. Transgenic animal technology including gene knockout technology, for example as a result of homologous recombination with an appropriate gene targeting vector, or gene overexpression, will result in the absence, decreased or increased expression of a polynucleotide or polypeptide. The same technology can also be applied to make knockout cells. When desired, tissue-specific expression or knockout of a polynucleotide or polypeptide may be necessary. Transgenic animals generated by such methods find use as animal models of mental illness and are useful in screening for modulators of mental illness.

[0081] Knockout cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into an endogenous gene site in the mouse genome via homologous recombination. Such mice can also be made by substituting an endogenous polynucleotide with a mutated version of the polynucleotide, or by mutating an endogenous polynucleotide, *e.g.*, by exposure to carcinogens.

[0082] For development of appropriate stem cells, a DNA construct is introduced into the nuclei of embryonic stem cells. Cells containing the newly engineered genetic lesion are injected into a host mouse embryo, which is re-implanted into a recipient female. Some of these embryos develop into chimeric mice that possess germ cells partially derived from the mutant cell line. Therefore, by breeding the chimeric mice it is possible to obtain a new line of mice containing the introduced genetic lesion (*see, e.g.*, Capecchi et al., Science 244:1288 (1989)). Chimeric targeted mice can be derived according to Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Labora-

tory (1988) and Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, Robertson, ed., IRL Press, Washington, D.C., (1987).

B. Modulators of polypeptides or polynucleotides

[0083] The modulators of the present invention are NCAM peptide mimetics. Modulators can be genetically altered versions of a polypeptide.

[0084] High throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (potential modulator or ligand compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[0085] A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

[0086] Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.*, U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al., Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g.*, PCT Publication No. WO 91/19735), encoded peptides (*e.g.*, PCT Publication WO

93/20242), random bio-oligomers (*e.g.*, PCT Publication No. WO 92/00091), benzodiazepines (*e.g.*, U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., Proc. Nat. Acad. Sci. USA 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara et al., J. Amer. Chem. Soc. 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., J. Amer. Chem. Soc. 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen et al., J. Amer. Chem. Soc. 116:2661 (1994)), oligocarbamates (Cho et al., Science 261:1303 (1993)), and/or peptidyl phosphonates (Campbell et al., J. Org. Chem. 59:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (*see, e.g.*, U.S. Patent 5,539,083), antibody libraries (*see, e.g.*, Vaughn et al., Nature Biotechnology, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries *(see, e.g.*, Liang et al., Science, 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.*, benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

[0087] Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.*, 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA; 433A Applied Biosystems, Foster City, CA; 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.*, ComGenex, Princeton, NJ; Tripos, Inc., St. Louis, MO; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, *etc.*).

C. Solid State and Soluble High Throughput Assays

[0088] In the high throughput assays, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential

modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (*e.g.*, 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 different compounds are possible using the integrated systems. More recently, microfluidic approaches to reagent manipulation have been developed.

[0089] The molecule of interest can be bound to the solid state component, directly or indirectly, via covalent or non-covalent linkage, *e.g.*, via a tag. The tag can be any of a variety of components. In general, a molecule that binds the tag (a tag binder) is fixed to a solid support, and the tagged molecule of interest is attached to the solid support by interaction of the tag and the tag binder.

[0090] A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, *etc.).* Antibodies to molecules with natural binders such as biotin are also widely available and appropriate tag binders *(see,* SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis MO).

[0091] Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody which recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs, such as agonists and antagonists of cell membrane receptors (*e.g.*, cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies,

the cadherin family, the integrin family, the selectin family, and the like; *see, e.g.*, Pigott & Power, The Adhesion Molecule Facts Book I (1993)). Similarly, toxins and venoms, viral epitopes, hormones (*e.g.*, opiates, steroids, *etc.*), intracellular receptors (*e.g.*, which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

[0092] Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

[0093] Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly-Gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to those of skill in the art. For example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc., Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

[0094] Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature (*see, e.g.,* Merrifield, J. Am. Chem. Soc. 85:2149-2154 (1963) (describing solid phase synthesis of, *e.g.*, peptides); Geysen et al., J. Immun. Meth. 102:259-274 (1987) (describing synthesis of solid

phase components on pins); Frank and Doring, Tetrahedron 44:60316040 (1988) (describing synthesis ofvarious peptide sequences on cellulose disks); Fodor et al., Science, 251:767-777 (1991); Sheldon et al., Clinical Chemistry 39(4):718-719 (1993); and Kozal et al., Nature Medicine 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

[0095] *In vitro* assays for identifying, in a high throughput format, compounds that can modulate the expression or activity of the polynucleotides or polypeptides disclosed herein are provided. The methods may include a control reaction. For each of the assay formats described, "no modulator" control reactions that do not include a modulator provide a background level of binding activity.

[0096] In some assays it will be desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. First, a known activator of a polynucleotide or polypeptide can be incubated with one sample of the assay, and the resulting increase in signal resulting from an increased expression level or activity of polynucleotide or polypeptide determined according to the methods herein. Second, a known inhibitor of a polynucleotide or polypeptide can be added, and the resulting decrease in signal for the expression or activity can be similarly detected.

D. Computer-Based Assays

[0097] Yet another assay for compounds that modulate the activity of a polypeptide or polynucleotide involves computer assisted drug design, in which a computer system is used to generate a three-dimensional structure of the polypeptide or polynucleotide based on the structural information encoded by its amino acid or nucleotide sequence. The input sequence interacts directly and actively with a pre-established algorithm in a computer program to yield secondary, tertiary, and quaternary structural models of the molecule. Similar analy-

ses can be performed on potential receptors or binding partners of the polypeptides or polynucleotides. The models of the protein or nucleotide structure are then examined to identify regions of the structure that have the ability to bind, *e.g.*, a polypeptide or polynucleotide. These regions are then used to identify polypeptides that bind to a polypeptide or polynucleotide.

[0098] The three-dimensional structural model of a protein is generated by entering protein amino acid sequences of at least 10 amino acid residues or corresponding nucleic acid sequences encoding a potential receptor into the computer system. The amino acid sequences encoded by the nucleic acid sequences provided herein represent the primary sequences or subsequences of the proteins, which encode the structural information of the proteins. At least 10 residues of an amino acid sequence (or a nucleotide sequence encoding 10 amino acids) are entered into the computer system from computer keyboards, computer readable substrates that include, but are not limited to, electronic storage media (*e.g.*, magnetic diskettes, tapes, cartridges, and chips), optical media (*e.g.*, CD ROM), information distributed by internet sites, and by RAM. The three-dimensional structural model of the protein is then generated by the interaction of the amino acid sequence and the computer system, using software known to those of skill in the art.

[0099] The amino acid sequence represents a primary structure that encodes the information necessary to form the secondary, tertiary, and quaternary structure of the protein of interest. The software looks at certain parameters encoded by the primary sequence to generate the structural model. These parameters are referred to as "energy terms," and primarily include electrostatic potentials, hydrophobic potentials, solvent accessible surfaces, and hydrogen bonding. Secondary energy terms include van der Waals potentials. Biological molecules form the structures that minimize the energy terms in a cumulative fashion. The computer program is therefore using these terms encoded by the primary structure or amino acid sequence to create the secondary structural model.

[0100] The tertiary structure of the protein encoded by the secondary structure

is then formed on the basis of the energy terms of the secondary structure. The user at this point can enter additional variables such as whether the protein is membrane bound or soluble, its location in the body, and its cellular location, *e.g.*, cytoplasmic, surface, or nuclear. These variables along with the energy terms of the secondary structure are used to form the model of the tertiary structure. In modeling the tertiary structure, the computer program matches hydrophobic faces of secondary structure with like, and hydrophilic faces of secondary structure with like.

[0101] Once the structure has been generated, potential ligand binding regions are identified by the computer system. Three-dimensional structures for potential ligands are generated by entering amino acid or nucleotide sequences or chemical formulas of compounds, as described above. The three-dimensional structure of the potential ligand is then compared to that of a polypeptide or polynucleotide of the invention to identify binding sites of the polypeptide or polynucleotide. Binding affinity between the protein and ligands is determined using energy terms to determine which ligands have an enhanced probability of binding to the protein.

[0102] Computer systems are also used to screen for mutations, polymorphic variants, alleles and interspecies homologs of genes encoding a polypeptide or polynucleotide. Such mutations can be associated with disease states or genetic traits and can be used for diagnosis. As described above, GeneChip™ and related technology can also be used to screen for mutations, polymorphic variants, alleles and interspecies homo logs. Once the variants are identified, diagnostic assays can be used to identify patients having such mutated genes. Identification of the mutated a polypeptide or polynucleotide involves receiving input of a first amino acid sequence of a polypeptide (or of a first nucleic acid sequence encoding a polypeptide), *e.g.*, any amino acid sequence having at least 60%, optionally at least 70% or 85%, identity with the amino acid sequence of interest, or conservatively modified versions thereof. The sequence is entered into the computer system as described above. The first nucleic acid or amino acid sequence is then compared to a second nucleic acid or amino acid

sequence that has substantial identity to the first sequence. The second sequence is entered into the computer system in the manner described above. Once the first and second sequences are compared, nucleotide or amino acid differences between the sequences are identified. Such sequences can represent allelic differences in various polynucleotides, and mutations associated with disease states and genetic traits.

V. Administration and Pharmaceutical compositions

[0103] Modulators of the polynucleotides or polypeptides disclosed herein (*e.g.*, antagonists or agonists, such as FGF2, NCAM, peptide inhibitors of the FGF system, or siRNA and/or antisense inhibitors of genes which are overexpressed in subjects with mental disorders) can be administered directly to a mammalian subject for modulation of activity of those molecules *in vivo.* Administration is by any of the routes normally used for introducing a modulator compound into ultimate contact with the tissue to be treated and is well known to those of skill in the art. Although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

[0104] Diseases that can be treated include the following, which include the corresponding reference number from Morrison, DSM-IV Made Easy, 1995: Schizophrenia, Catatonic, Subchronic, (295.21); Schizophrenia, Catatonic, Chronic (295.22); Schizophrenia, Catatonic, Subchronic with Acute Exacerbation (295.23); Schizophrenia, Catatonic, Chronic with Acute Exacerbation (295.24); Schizophrenia, Catatonic, in Remission (295.55); Schizophrenia, Catatonic, Unspecified (295.20); Schizophrenia, Disorganized, Subchronic (295.11); Schizophrenia, Disorganized, Chronic (295.12); Schizophrenia, Disorganized, Subchronic with Acute Exacerbation (295.13); Schizophrenia, Disorganized, Chronic with Acute Exacerbation (295.14); Schizophrenia, Disorganized, in Remission (295.15); Schizophrenia, Disorganized, Unspecified (295.10); Schizophrenia, Paranoid, Subchronic (295.31); Schizophrenia, Paranoid, Chronic (295.32); Schizophrenia, Paranoid, Subchronic with Acute Exa-

cerbation (295.33); Schizophrenia, Paranoid, Chronic with Acute Exacerbation (295.34); Schizophrenia, Paranoid, in Remission (295.35); Schizophrenia, Paranoid, Unspecified (295.30); Schizophrenia, Undifferentiated, Subchronic (295.91); Schizophrenia, Undifferentiated, Chronic (295.92); Schizophrenia, Undifferentiated, Subchronic with Acute Exacerbation (295.93); Schizophrenia, Undifferentiated, Chronic with Acute Exacerbation (295.94); Schizophrenia, Undifferentiated, in Remission (295.95); Schizophrenia, Undifferentiated, Unspecified (295.90); Schizophrenia, Residual, Subchronic (295.61); Schizophrenia, Residual, Chronic (295.62); Schizophrenia, Residual, Subchronic with Acute Exacerbation (295.63); Schizophrenia, Residual, Chronic with Acute Exacerbation (295.94); Schizophrenia, Residual, in Remission (295.65); Schizophrenia, Residual, Unspecified (295.60); Delusional (Paranoid) Disorder (297.10); Brief Reactive Psychosis (298.80); Schizophreniform Disorder (295.40); Schizoaffective Disorder (295.70); Induced Psychotic Disorder (297.30); Psychotic Disorder NOS (Atypical Psychosis) (298.90); Personality Disorders, Paranoid (301.00); Personality Disorders, Schizoid (301.20); Personality Disorders, Schizotypal (301.22); Personality Disorders, Antisocial (301.70); Personality Disorders, Borderline (301.83) and bipolar disorders, maniac, hypomaniac, dysthymic or cyclothymic disorders, substance-induced mood disorders, major depression, psychosis, including paranoid psychosis, catatonic psychosis, delusional psychosis, having schizoaffective disorder, and substance-induced psychotic disorder.

[0105] Modulators of polynucleotides or polypeptides may be combined with other drugs useful for treating mental disorders including useful for treating mood disorders, *e.g.*, schizophrenia, bipolar disorders, or major depression. Pharmaceutical compositions may comprise a modulator of a polypeptide or polynucleotide combined with at least one of the compounds useful for treating schizophrenia, bipolar disorder, or major depression, *e.g.*, such as those described in U.S. Patent Nos. 6,297,262; 6,284,760; 6,284,771; 6,232,326; 6,187,752; 6,117,890; 6,239,162 or 6,166,008.

[0106] The pharmaceutical compositions of the invention may comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are

determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g.*, Remington's Pharmaceutical Sciences, 17th ed. 1985)).

[0107] The modulators (*e.g.*, agonists or antagonists) of the expression or activity of the a polypeptide or polynucleotide, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be "nebulized") to be administered via inhalation or in compositions useful for injection. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0108] Formulations suitable for administration include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, orally, nasally, topically, intravenously, intraperitoneally, or intrathecally. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials. Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. The modulators can also be administered as part of a prepared food or drug.

[0109] The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial response in the subject over time. The optimal dose level for any patient will depend on a variety of factors including the efficacy of the specific modulator employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the mental disorder. The size of the dose also will be determined by the existence, nature, and extent of any adverse side effects

that accompany the administration of a particular compound or vector in a particular subject.

[0110] In determining the effective amount of the modulator to be administered a physician may evaluate circulating plasma levels of the modulator, modulator toxicity, and the production of anti-modulator antibodies. In general, the dose equivalent of a modulator is from about 1 ng/kg to 10 mg/kg for a typical subj ect.

[0111] For administration, modulators of the present invention can be administered at a rate determined by the LD-50 of the modulator, and the side effects of the modulator at various concentrations, as applied to the mass and overall health of the subject. Administration can be accomplished via single or divided doses.

Example 1: Differential expression of genes associated with suicide in both BP and MDD subjects

[0112] Previous studies have investigated genes associated with mood disorders and suicidal tendencies, using microarrays and PCRs to analzye gene expression (Sibille *et al.,* 2004; Yanagi M, et al., J Hum Genet., 50(4):210-6(2005)). Neither investigation, however, used a stringent analysis of suicide compared to mood disorder and suicide compared to controls to detect genes that might be most representative of suicide. This Example describes microarray gene expression profiles in the amygdala, anterior cingulate, and cerebellum in postmortem brains from BPD and MDD patients that committed suicide, focusing on mRNA expression levels of the molecules which regulate white-matter, oligodendrocyte, myelin, and other pathways. The genes identified here may be used as biomarkers for detecting and treating suicidal behavior.

[0113] Genes were discovered by selecting subjects run on U133P chips with mRNA quality > 1.4, pH>6.6, and AFS=0. Suicide victims with a mood disorder (n = 14) were compared to non-suicide victims suffering from a mood disorder

(n =9) and controls (n =27). The age and pH were different between groups, and were entered as a covariate in ANCOVA. Myelin and oligodendrocyte gene expression were found to be dysregulated in suicidal mood disorder subjects compared to non-suicidal mood disorder subjects or controls in the amygdala. The complete list of identified genes which were dysregulated in suicidal patients versus non-suicidal mood disorder patients is presented in Table 1A. Table 1B lists genes which were dysregulated in suicidal MDD patients versus non-suicidal MDD patients, and genes which were dysregulated in suicidal MDD patients versus control patients.

[0114] A similar study was performed using brains of MDD subjects who were known to be drug abusers and comparing gene expression in those subjects to gene expression in MDD subjects who were not substance abusers, as well as to control subjects. Table 1C is a list of genes which were shown to be dysregulated in substance-abusing MDD patients versus MDD patients who were not substance abusers. Table 1C also shows genes which were dysregulated in substance-abusing MDD patients versus control subjects.

[0115] In a related study, two cohorts were used to study and compare gene expression in BP and MDD patients versus normal patients. Cohort A consisted of 7 controls, 6 BPD patients, and 9 MDD patients. Cohort B included 7 controls and 5 MDD patients. The subjects were selected to avoid possible confounding effects of agonal events, tissue pH, RNA integrity, gender and age. The results, summarized in Figures 5-8, show that changes were observed in the expression levels of GPCRs and molecules regulating cAMP-and phosphatidylinositol signaling pathways in the cerebral cortices, especially in the anterior cingulate cortex, of mood disorder patients. Expression levels of molecules acting as negative regulators in cAMP signaling were increased in BPD, while molecules activating cAMP signaling were not altered. Contrasted with the changes in BPD, molecules suppressing cAMP signaling were decreased in MDD. Expression levels of inositol polyphosphate-1-phosphatase and phosphatidylinositol 3-kinases were altered in BPD, while protein kinase C beta-1, inositol triphosphate receptor-1, inositol polyphosphate-5-phosphatase were increased in MDD. Two

orphan GPCR genes, GPRC5B and GPR37, consistently showed significant decreases in the three cortices in MDD, and significant increases in anterior cingulate cortex of BPD. Measuring differences in the expression of the genes identifed in Figures 5-8 is a useful tool for determining whether a subject is suffering from a particular mental illness, particularly BP or MDD.

Example 2: Identification of lithium responsive genes which are dysregulated in BPD

[0116] This Example demonstrates that certain genes in non-human primates (healthy rhesus macaque monkey) are differentially expressed in response to treatment with the mood-stabilizing drug, lithium (Li), the drug of choice for the treatment of BP. Gene expression profiling was carried out on the anterior cingulate cortex (AnCg), dorsolateral prefrontal cortex (DLPFC), hippocampus (HC) and amygdala (AMY) of rhesus macaque monkeys, using the gene expression detection methods described herein, and compared to the human postmortem results described above. Table 2A shows the lithium-responsive genes which had been previously identified in the literature and which were confirmed by the present investigation. Table 2B shows genes that are newly identified as lithium-responsive in primates and which are also dysregulated in human subjects with bipolar disorder.

Example 3: FGFR2 variant differences in Mood Disorders.

[0117] The FGF receptor 2 (FGFR2) transcript is consistently found to be decreased in several brain areas of depressed subjects (*see, e.g.*, U.S. Pat. App. No. 10/701,263, filed Nov. 3, 2003, published as U.S. Pat. Publ. No. 2004-0152111-A1 on August 5, 2004). The human FGFR2 gene contains 19 exons and produces as many as 13 splice variants. These variants fall into three main functional classes: first, variants that lack the transmembrane and tyrosine kinase domain which are thought to be soluble receptors; second, variants that contain the Ig IIIc type domain encoded by exon 9; and third, variants that contain the Ig IIIb type domain encoded by exon 8. The Ig III type domain confers

ligand specificity and thus these latter two variants have different pharmacological profiles based on their use of the IIIc or IIIb domain. This Example describes PCR-based measurements of exons present in total RNA derived from human cortex (dorsolateral prefrontal and anterior cingulated) and hippocampus.

[0118] Methods Post-mortem human brans were obtained and dissected as previously described (Evans et al., PNAS 101(43):15506-11 (2004)). RNA for microarray analysis and semi-quantitative RT-PCR was extracted from discrete brain regions using Trizol.

[0119] Microarray data was generated with a combination of Affymetrix 133A anti 133plus 2.0 chips and was analyzed using a custom probe mapping file based on a recent generation of the RefSeq database (http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF). Each biological sample was run independently at two sites (University of California-Irvine, University of run California-Davis or the University of Michigan). Probe set signals were calculated using RMA (Bolstad et al., Bioinformatics 19(2):185-93 (2003)) and statistical comparisons were made after median centering the RMA data separately for each technical block (across independent cohorts and sites). P-values were constructed from t-tests between cases and controls. The final subject composition included 13 major depressive subjects and 16 controls. All were free of agonal factors, had brain pH measurements greater than 6.8, and met other quality measures.

[0120] Semi-quantitative RT-PCR data were generated with exon specific primers and the SVBR green method using the BioRad iCycler. All primer pairs used in quantitative reactions were tested for efficiency and determined to be at approximately 100%. Cycle threshold (Ct) values were chosen within the linear range of amplification and were normalized to total cDNA concentration as determined by the PicoGreen assay (Molecular Probes). Contaminating genomic DNA was eliminated with DNAse prior to cDNA synthesis with a mixture of random hexamers and poly-T primers and was confirmed eliminated by amplifying

fragments across smaller introns (axon 3 to exon 4 and exon 7 to exon 10). No intron-containing amplicons were detected.

[0121] Results The results of the above-mentioned study are partially summarized in FIGURE 1, which shows the differential expression of exons 5 and 11 in depressed versus control subjects. More specifically, the results show that the ratio of expression of exon 5 to exon 11 is significantly lowered in MDD patients, particularly in the DLPFC region. A similar analysis of the expression of exon 9 (coding for the IIIc variant) showed that exon 9 expression in the AnCg and HC regions was decreased in MDD subjects.

Example 4: Effect of injection of FGF2, FGL peptide (NCAM), and peptide inhibitor of FGF receptors on the behavior of rodents

A. Microinjection of FGF2

[0122] This set of experiments shows significant effects following the microinjection of FGF2, using both the forced swim test (FST) and the elevated plus maze (EPM) to evaluate depression in the subject animals. In the FST, the FGF2-injected (n = 12) animals exhibited more swimming ($t[23] = 2.20$, $p < 0.05$) and less immobility ($t[23] = 2.88$, $p<0.01$) than controls (n = 13). This is indicative of less depression-like behavior in FGF2 animals. However, in the EPM, the FGF2-injected animals spent significantly more time in the closed arms ($t[13] = 3.18$, $p<0.01$) and less time in the open arms ($t[13] = 2.46$, $p < 0.05$). These results (FIGURE 2) show that anxiety-like behavior is increased after an acute injection of FGF2.

B. Microinjection of NCAM (FGL peptide)

[0123] This set of experiments shows a significant effect on animal mood in the forced swim test after NCAM administration (amino acid sequence = EVYVVAENQQGKSKA; *see* FIGURE 3). Here, the NCAM-injected animals (n = 13) exhibited less immobility ($t[24] = 2.13$, $p < 0.05$) than controls (n = 13).

Again, this is an index of less depression-like behavior. This is also in the same direction as the FGF2 data, consistent with the fact that both FGF2 and NCAM interact with the FGF receptor. Similarly, the NCAM-injected animals spent more time (although not signficantly more time) in the closed arms and less time in the open arms, consistent with increased anxiety-like behavior. NCAM-injected animals also spent significantly less time in the center quadrant ($t[18] = 2.40$, $p < 0.05$).

C. Microinjection of a peptide inhibitor of FGFR

[0124] This set of experiments shows a significant effect on animal mood, using both the forced swim test and the elevated plus maze test, after injection with an FGF system peptide inhibitor (amino acid sequence = HFKDPKRLY). The results are shown in FIGURE 4. In the FST, the inhibitor-injected animals (n=7) exhibited significantly less climbing ($t[12] = 2.06$, $p < 0.05$), less swimming ($t[12] = 1.92$, $p <0.05$) and more immobility ($t[12] = 3.58$, $p < 0.005$) than controls (n = 7). These results show that inhibition of the FGF system can result in increased depression-like behavior. These results confirm and advance the results of the previous data sets, and are consistent with studies of the postmortem tissue of individuals with major depression. The inhibitor-injected animals also spent significantly more time in the center quadrant of the EPM ($T[7,7] = 35.0$, $p = 0.03$). Although the same animals spent less time in the closed arms and equal time in the open arms, indicative of increased anxiety-like behavior, the lengths of time spent were not significantly different. These observations are nevertheless consistent with the conclusions drawn from the microinjection studies above.

D. Administration of FGF2 induces long-term changes in hippocampal gene expression

[0125] Methods. Sprague- Dawley rats were injected with either vehicle or FGF2 (20ng/g, s.c.) the day after birth and sacrificed after Morris water maze testing as adults. We assessed changes in gene expression using both a can-

didate approach and a gene discovery approach with laser-capture microdissection of the dentate gyrus followed by microarray analyses.

[0126] Results. Rats injected with FGF2 performed significantly better in learning and memory tests (*e.g.*, 20 seconds on average to find a hidden platform in Morris Water maze test versus 25 seconds on average for vehicle-injected rats). Several genes associated with neural plasticity were also found altered in the adult rats, as shown by histochemical and RNA expression assays. For example, expression of GAP-43, Rgs4, trkB, CCK, SST, and Vgfwas increased, while expression of NCAM was decreased.

Example 5: Anxiolytic Effect of Chronically Administered FGF2

[0127] Anxiety disorders have a high comorbidity with other neuropsychiatric disorders including Major Depression (MD). This Example shows that chronic FGF2 administration has an anxiolytic effect in rats. Rats were placed into "high anxiety" (LR) or "low anxiety" (HR) groups based on their behavior in a variety of motor and behavioral tests. Both groups of animals were administered either FGF2 (5 ng/g) or vehicle by intraperitoneal injection every 48 hours for 3 weeks. One day after the last FGF2 injection, all animals were tested in the elevated plus-maze (EPM) and light-dark (LD) anxiety test.

[0128] The apparatus for the EPM test is constructed of black Plexiglass with four elevated open arms (70cm from the floor, 45cm long, and 12cm wide). Illumination is provided by a 40-watt desk lamp facing a wall and placed behind one of the closed arms. The scientists put the animal inside the system. Animals that are less anxious spend more time in the open arms whereas animals that are more anxious spend less time in the open arms.

[0129] The LD test is conducted in a 30 x 60 x 30-cm Plexiglas shuttle box with a translucent cover. Each box is divided into two equal-sized compartments by a wall with a 12 cm-wide open door. One compartment is painted white and brightly illuminated, and the other is painted black with very dim light. The time

each rat spends in each compartment is monitored by rows of five photocells located 2.5 cm above the grid floor of each compartment. Animals that are less anxious spend more time in the light compartment.

[0130] The results show that animals who received chronically administered FGF2 are less anxious than animals who receive vehicle (FIGURE 9, top). The anxiety-reducing effects of FGF2 are clearly more pronounced in rats who are inately more anxious (LR) prior to the FGF2 regimen (FIGURE 9, top).

[0131] To further understand the relationship between FGF2 expression and anxiety, FGF2 expression was measured in the CA-2 region of the hippocampus of rat brains taken from rats which exhibited varying levels of anxiety (as measured by the EPM test). The results (FIGURE 10) show that FGF2 levels are inversely related to anxiety, *i.e.*, higher levels of inate FGF2 expression in rats correlate with lower levels of anxiety.

[0132] Taken as a whole, the Example shows that chronic FGF2 administration is useful for alleviating symptoms of anxiety in anxious animals and in subjects who are suffering from disorders such as MDD which are associated with anxiety. The data also shows that detection of FGF2 levels is useful for diagnosing anxiety or characterizing disorders associated with anxiety, such as Major Depression Disorder.

Example 6: Differential regulation of FGFR splice variants associated with chronic stres

[0133] In the adult CNS, fibroblast growth factor receptor 2 (FGFR2) and fibroblast growth factor receptor 3 (FGFR3) are differentially distributed. The mRNA of these receptors undergoes alternative splicing in the exons coding for the carboxyl terminus of the Ig-like domain III. This mutually exclusive mRNA splicing produces two isoforms of FGFR2 and FGFR3 with significantly different ligand binding profiles: one isoform expressing exon IIIb (FGFR2b/FGFR3b), and one isoform expressing exon IIIc (FGFR2c/FGFR3c). Exon selection is

strictly tissue-dependent during development with exon IIIb expressed in epithelial lineages and exon IIIc expressed in mesenchymal lineages. Cell cycle-dependent IIIb to IIIc swiches, however, have been induced *in vitro* by the exogenous addition of FGF1 and FGF2. This Example shows that chronic stress induces a decrease in the FGFR2 exon IIIc:IIIb splice variant expression ratio.

[0134] Animals. Twenty-four male Sprague-Dawley rats weighing 220-250g were ordered from Charles River (Wilmington, MA) and remained undisturbed for one week to acclimatize to housing conditions. The animals were housed in pairs on a 12h light-dark cycle (lights on 6:00 A.M.) with food and water available *ad libitum*. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Animals and the University Committee on the Use and Care of Animals.

[0135] FGF2 injection treatments and chronic unpredictable stress (CUS) conditions. Half of the rats (n = 12) were administered vehicle (0.1M PBS with 100 µg/mL bovine serum albumin), and the other half (n = 12) were administered human recombinant FGF2 dissolved in vehicle in 5 ng/g dosages (Sigma, St. Louis, MO) every 48 hours for three weeks. All treatments were injected intra-peritonealy. During the same three week period as the FGF2 treatments, the vehicle group was either handled (n = 6) or exposed to CUS (n = 6). Likewise, the FGF2 injected group was either handled (n = 6) or exposed to CUS (n = 6). The animals were exposed to the following chronic unpredictable stressors (described in Isgor et al. (2004)): ether (30s), cold (2h), noise (15m), isolation (24h), or restraint (2h). The stressors were randomized to avoid habituation; sessions occurred once each day in either the morning or afternoon. The 2x2 (condition by treatment) design divided the subjects into nonstressed/vehicle (NS/V), nonstressed/FGF2 injection (NS/F), stressed/vehicle (S/V), and stressed/FGF2 injection (S/F) groups.

[0136] Forced swim testing. To test for possible FGF2 injection effects on antidepressant behavior for other studies, all animals were subjected to forced

swim testing according to Lucki (1997) 24h after the termination of the FGF2 treatments and CUS conditions.

[0137] Brains. The rats were sacrificed by the faculty by decapitation three days after termination of the forced swim testing. Brains were then removed and snap frozen in isopentane at -80°C.

[0138] Total RNA extraction. Gross dissections were performed on the frontal cortex of all brains. Total RNA extraction was executed following the reagent manufacturers' instructions. Tissues were homogenized in TRIzol (Invitrogen, Carlsbad, CA; monophasic phenol and guanidine isothiocyanate). Phase separation and RNA precipitation were carried out with chloroform and 2-propanol followed by centrifugation. RNA samples were purified using the RNeasy Mini Kit (Qiagen, Valencia, CA), repeatedly washing samples through spin columns. Pure RNA samples were reconstituted with RNase-free water, followed by an integrity analysis for 28s/18s ribosomal RNA peaks with the 2100 Bioanalyzer and LabChip (Agilent Technologies, Palo Alto, CA) system. Using the Bioanalyzer's concentration readings, the samples were normalized to 25 ng/μL of total RNA per subject and stored at -80°C.

[0139] cDNA synthesis. cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). All 24 RNA samples were reversed transcribed with iScript Reverse Transcriptase (Bio-Rad) and primed with oligo(dT) and random hexamer primers. Reaction mixes were incubated in an iCycler PCR unit (Bio-Rad) according to the manufacturer's standard 40min protocol. The double-stranded cDNA solutions were then analyzed for quality and concentration using Invitrogen's Quant-iT PicoGreen dsDNA kit. 10-fold serial dilutions were prepared with 2 μg/mL (high range) and 50 ng/mL (low range) stock DNA to generate standard curves. The fluorescently labeled samples were analyzed with a 1420 Victor^2 Multilabel Counter (EG&G Wallac, Wellesley, MA) using the basic Fluorescein protocol. Total cDNA samples were further normalized to fit the linear regression of the high range standard curve.

[0140] Real time RT-PCR primer design. Sequences for rat FGFR2 and FGFR3 exons were obtained from NCBI's Entrez Gene database (www.ncbi.nlm.nih.gov) and the Ensembl-Rat gene database (www.ensembl.org). Exon sequences were analyzed using NCBI's nucleotide and protein BLAST and were matched with their corresponding protein products within the receptor structures (FIGURE 11). FGFR2 and FGFR3 exon sequences were analyzed for secondary structure using the Mfold nucleic acid folding web server. Probable hairpin regions were noted for exclusion in primer design. Optimized primer sequences (FIGURE 11) were generated using the Primer3 web-based software. All primers were 18-22 base pairs, targeted amplicons of 75-150 bps, and purchased from Invitrogen's Custom Primer Synthesis service. The primer sequences (50 nmol/mL) were validated and tested for efficiency with 5-fold serial dilutions of pooled cDNA using iQ SYBR Green detection on an iCycler iQ Real Time RT-PCR unit (Bio-Rad).

[0141] Real time RT-PCR quantification. Real time reverse-transcriptase-PCR (RT-PCR) amplification reactions were performed to quantify relative abundances of mRNA (reverse transcribed to cDNA) of selected FGFR2 and R3 exons in each of the four treatment by condition groups (n=6). iQ SYBR Green Supermix detection was used on an iCycler iQ Real Time RT-PCR system (Bio-Rad) according to the manufacturer's recommendations, with the exception of preparing 19µL reactions instead of the instructed 50µL. Reference genes were omitted because total cDNA pools were normalized. Reactions were run in duplicates, increasing each group size to n=12. Two exons in juxtaposition were amplified per plate for relative comparison (emphasis placed on exon IIIb and exon IIIc). Reaction wells were arranged to equalize positional representation amongst all groups. PCR protocol was as follows: hot start at 95°C for 30s, followed by 40 cycles of denature at 95°C for 15s, annealing at 60°C for 15s, and elongation at 72°C for 15s. Florescence was quantified after every cycle, and melt curve analysis was performed following amplification to ensure single product reactions. Thorough methodology is described by Kerman et al. (2006).

[0142] Data analysis Real time RT-PCR data was output as threshold cycle

(Ct) values, using Bio-Rad's iCycler iQ software's algorithm to calculate the optimum fluorescence thresholds for reliable detection (the mean florescence value of the first ten PCR cycles plus 10 standard deviations). Essentially, lower Ct values indicate higher amounts of initial target cDNA because fewer PCR cycles are required to reach fluorescence thresholds. Ct values for all reactions were then grouped and presented as means with standard errors. Sample sizes were 9-12 per group due to outlier (≥2 StDev from mean) exclusion. Mean fold changes were calculated using a $2^{\Delta Ct}$ method (modification of technique described by Livak and Schmittgen (2001)), comparing mean Ct values within one variable (treatment effects within one condition or condition effects within one treatment). This $2^{\Delta Ct}$ method assumes equal primer efficiencies; however, for the purposes of quantifying relative expression, it is acceptable if the primers are validated. Exon IIIc to exon IIIb ratios were determined by calculating individual $2^{\Delta Ct}$ values between corresponding reactions of the two exons. Ratios were sorted similarly to the Ct value groups and presented as mean ratios with standard errors. Statistical significance tests for differences in multiple exon mean Ct values and individual IIIc:IIIb ratios were performed using two-factor ANOVA and Student's *t-test* for each comparison variable. Significance level was set as $p < 0.05$. All statistical analysis was done in Microsoft Excel 2003.

[0143] Results. Chronic stress induced significant decreases in the exon IIIc to exon IIIb (mutually exclusive expression) splice variant ratio in the vehicle group ($P<0.00004$) and in the FGF2 injection group ($P<0.005$) (FIGURE 12). While exon IIIc expression remained relatively higher than IIIb in all groups, IIIb expression increased significantly with stress while IIIc expression changed only slightly. Thus, stress increases expression of the IIIb variant relative to the IIIc variant for both FGFR2 and FGFR3. FGF2 injection did not alter the IIIc:IIIb ratio significantly in either the NS or S group ($P>0.1$), nor did it significantly affect the magnitude of IIIc:IIIb ratio changes caused by stress.

[0144] The results show that the affinity of FGFR2 or FGFR3 for endogenous ligands such as FGF2 and FGF9 (which are differentially expressed in MDD subjects) or for exogenous ligands (*e.g.*, pharmacological peptides or other

compounds) can be altered by stress. The invention described herein provides methods of detecting variations in FGFR2 and FGFR3 splicing and modifying subject care accordingly. In another embodiment, the invention provides methods for identifying and optimizing therapeutics for treating depression and related ailments.

Example 7: Differential regulation of genes in the Locus Coeruleus and the Dorsal Raphe in subjects with bipolar and major depression disorder

[0145] The Locus Coeruleus (LC) and the Dorsal Raphe (DR) are the major sources for noradrenergic and serotonergic innervation of the brain respectively. Dysregulation of these neurotransmitters has been implicated in psychiatric disorders. This Example uses postmortem brains of patients with MDD (N=12), BPD (N=6), and healthy subjects (N=9) to contrast gene expression profiles in the LC and DR regions of their brains. All subjects met inclusion criteria of brain pH > 6.6 and zero agonal factors. Total RNA samples from laser capture microdissected LC and DR samples were extracted, amplified, and probed with Affymetrix high density oligonucleotide microarrays. Gene expression data were analyzed by ANOVA of robust multichip average algorithm ($p \leq 0.1$) and by MAS5.0 "present" call algorithm (min.of 50% presences in one of the 3 health states). Genes meeting these criteria were analyzed using Ingenuity Pathway Analysis (IPA). Compared to healthy individuals, 774 and 636 genes show altered expression in the LC and 627 and 656 genes show altered expression in the DR of MDD and BPD patients, respectively.

[0146] LC gene expression patterns: The data is summarized in Table 3. Ingenuity Pathway Analysis revealed that 10 genes of the glutamate receptor signaling pathway are significantly altered in MDD ($p<0.01$) but not in BPD. Glutamate signaling gene expression alterations are present in following synaptic compartments of the locus coeruleus: glial cells, presynpatic neurons, and postsynaptic neurons. This shows that glutamate signaling is altered in LC of MDD patients. Glial transporters, glutamine synthetase, AMPA, kainate, GRM1 and GRM7 are thus targets for treating glutamatergic imbalance.

[0147] The expression of genes related to growth, *i.e.*, fibroblast growth factors, are also significantly altered in the LC of MDD patients. Drugs that target FGFR3, TrkB receptor, growth hormone receptor, or which mimic the actions of FGF-2, would increase neurite outgrowth in the LC and reserve neuronal loss.

[0148] Genes that are almost exclusively expressed in glia are significantly downregulated. These genes are useful markers for global glial alterations in MDD patients.

[0149] DR gene expression patterns. The data is summarized in Table 4. IPA analyses of the DR revealed alterations in the expression of a number of genes in growth factor-related pathways in MDD. Expression of most of the altered genes was downregulated. Likewise, the expression of a number of genes in growth factor-related pathways was downregulated in samples from the BP cohort. However, these genes were distinct from those detected in the MDD cohort. Several genes that were common to both disorders were identified; their expression was altered in the same direction in MDD and BPD subjects.

TABLE 1A

Genes associated with suicide.										
Symbol	**Suicide dysregulation**	**Name**	**Best Accession**	**Cytoband**	**Anterior Cingulate Fold Change**		**Amygdala Fold Change**		**Cerebellum Fold Change**	
					Suicide Vs Mood	**Suicide Vs Control**	**Suicide Vs Mood**	**Suicide Vs Control**	**Suicide Vs Mood**	**Suicide Vs Control**
FOS	3 region	V-fos FBJ murine osteosarcoma viral oncogene homolog	BM894421	14q24.3	0.671	0.679	0.716	0.638	0.770	0.659
CAPN	2 region	"Calpain 3, (p94)"	BC003	15q15	0.78	0.79	0.56	0.65	0.84	0.92

Genes associated with suicide.

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change		Amygdala Fold Change		Cerebellum Fold Change	
					Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control
3			169	.1-q21.1	3	9	7	1	6	6
FOS	2 region	V-fos FBJ murine osteosarcoma viral oncogene homolog	BM894421	14q24.3	0.671	0.679	0.716	0.638	0.770	0.659
CAPN3	ancng	"Calpain 3, (p94)"	BC003169	15q15.1-q21.1	0.783	0.799	0.567	0.651	0.846	0.926
FGF12	ancng	Fibroblast growth factor 12	BC022524	3q28	1.393	1.303	1.060	1.167	0.896	1.074
FOS	ancng	V-fos FBJ murine osteosarcoma viral oncogene homolog	BM894421	14q24.3	0.671	0.679	0.716	0.638	0.770	0.659
MAP2	ancng	Microtubule-associated protein 2	AL535786	2q34-q35	1.389	1.303	0.914	1.084	0.921	0.999
LRIG1	ancng	Leucine-rich repeats and immunoglobulin-like domains 1	AI459030	3p14	0.799	0.689	1.008	0.897	0.965	0.810
TENS1	ancng	Tensin-like SH2 domain containing 1	CN480168	7p13-p12.3	0.704	0.737	0.992	0.854	0.953	0.721
ADAMTS1	ancng	"A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1"	CA446773	21q21.2	0.796	0.721	0.941	0.649	1.014	0.822

Genes associated with suicide.

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change		Amygdala Fold Change		Cerebellum Fold Change	
					Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control
CNTN1	ancng	Contactin 1	U07819	12q11-q12	1.320	1.283	1.038	1.098	0.883	0.994
GABRG2	ancng	"Gamma-aminobutyric acid (GABA) A receptor, gamma 2"	BI754570	5q31.1-q33.1	**1.376**	1.282	**0.902**	1.236	0.858	1.028
EFEMP1	ancng	EGF-containing fibulin-like extracellular matrix protein 1	CR611721	2p16	**0.791**	**0.749**	**0.784**	0.826	**0.979**	0.787
TPBG	ancng	Trophoblast glycoprotein	CA413562	6q14-q15	**1.495**	1.334	**1.057**	1.323	1.029	1.025
C3orf4	amygdala	Chromosome 3 open reading frame 4	CD102473	3p11-q11	0.897	**0.909**	**0.705**	0.763	0.903	0.921
EVI2A	amygdala	Ecotropic viral integration site 2A	CA415486	17q11.2	0.883	**0.862**	**0.669**	0.714	0.899	0.884
CD9	amygdala	CD9 antigen (p24)	AW864408	12p13.3	0.940	**0.850**	**0.760**	**0.798**	**0.821**	**0.702**
MOG	amygdala	Myelin oligodendrocyte glycoprotein	NM_002433	6p22.1	**0.847**	**0.792**	**0.644**	0.711	0.905	0.883
CAPN3	amygdala	"Calpain 3, (p94)"	BC003169	15q15.1-q21.1	**0.783**	**0.799**	**0.567**	0.651	0.846	0.926
UGT8	amygdala	UDP glycosyltransferase 8 (UDP-	CN275924	4q26	**0.945**	**0.781**	**0.710**	0.776	0.926	0.947

Genes associated with suicide.										
Symbol	**Suicide dysregulation**	**Name**	**Best Accession**	**Cytoband**	**Anterior Cingulate Fold Change**		**Amygdala Fold Change**		**Cerebellum Fold Change**	
					Suicide Vs Mood	**Suicide Vs Control**	**Suicide Vs Mood**	**Suicide Vs Control**	**Suicide Vs Mood**	**Suicide Vs Control**
		galactose ceramide galactosyltransferase)								
ASPA	amygdala	"Aspartoacylase (aminoacylase 2, Canavan disease)"	BF115120	17pter-p13	**0.903**	**0.816**	**0.761**	0.763	0.910	0.923
PLP1	amygdala	"Proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)"	BP194315	Xq22	0.935	**0.888**	**0.765**	0.751	0.876	0.848
ENPP2	amygdala	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	CR606785	8q24.1	**0.816**	**0.817**	**0.602**	**0.717**	0.813	**0.851**
ENPP2	amygdala	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	CR606785	8q24.1	**0.816**	**0.817**	**0.602**	**0.717**	0.813	**0.851**
RPH3A	amygdala	Rabphilin 3A homolog (mouse)	CR613722	12q24.13	1.241	**1.176**	**1.259**	1.283	0.994	1.095
SLC31A2	amygdala	"Solute carrier family 31 (copper transporters), member 2"	NM_001860	9q31-q32	0.875	**0.864**	**0.707**	0.798	0.957	0.966
FOS	amygda-	V-fos FBJ murine	BM894	14q24	**0.67**	**0.67**	**0.71**	**0.63**	**0.77**	**0.65**

Genes associated with suicide.

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change		Amygdala Fold Change		Cerebellum Fold Change	
					Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control
	la	osteosarcoma viral oncogene homolog	421	.3	**1**	**9**	**6**	**8**	**0**	**9**
CNP	amygdala	"2',3'-cyclic nucleotide 3' phosphordiesterase"	CD369491	17q21	0.836	**0.838**	**0.754**	0.761	0.910	0.872
SEPP1	amygdala	"Selenoprotein P, plasma, 1"	BF941781	5q31	**0.867**	**0.771**	**0.733**	0.785	**0.842**	0.667
PMP22	amygdala	Peripheral myelin protein 22	BG939651	17p12-p11.2	**0.845**	**0.772**	0.793	0.793	**0.927**	0.779
GREM1	amygdala	"Gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis)"	NM_013372	15q13-q15	0.827	**0.912**	**0.800**	0.798	0.970	0.991
GPR37	amygdala	G protein-coupled receptor 37 (endothelin receptor type B-like)	BF966147	7q31	0.991	**0.878**	**0.735**	0.784	0.954	0.917
HSPA2	amygdala	Heat shock 70kDa protein 2	BM979297	14q24.1	1.008	**0.851**	**0.702**	0.724	0.908	0.883
CD74	amygdala	"CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)"	BI262425	5q32	**0.806**	**0.766**	**0.787**	0.746	**0.895**	0.738
FA2H	amygda-	Fatty acid 2-	AA454	16q23	0.89	**0.87**	**0.68**	0.73	0.90	0.92

Genes associated with suicide.

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change		Amygdala Fold Change		Cerebellum Fold Change	
					Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control	Suicide Vs Mood	Suicide Vs Control
	la	hydroxylase	978		8	**5**	**5**	4	6	2
C20orf35	amygdala	Chromosome 20 open reading frame 35	B1711408	20q13.12	0.865	**0.902**	**0.759**	0.750	0.930	0.868
ZFYVE16	amygdala	"Zinc finger, FYVE domain containing 16"	AK127003	5p15.2-q14.3	**0.862**	**0.797**	**0.772**	0.776	0.868	0.992
MAG	amygdala	Myelin associated glycoprotein	X98405	19q13.1	**0.870**	**0.829**	**0.685**	0.726	1.011	0.904
SATB2	amygdala	SATB family member 2	AW970253	2q33	1.231	**1.188**	**1.549**	1.302	0.975	1.019
TF	amygdala	Transferrin	CB156966	3q22.1	**0.890**	**0.829**	**0.669**	0.711	0.878	0.881
OPN3	amygdala	"Opsin 3 (encephalopsin, panopsin)"	BU631266	1q43	1.257	**1.151**	**1.293**	1.343	1.081	1.044
CGI-38	amygdala	Brain specific protein	BM981844	16q22.1	1.000	**1.000**	**1.667**	1.563	1.000	1.000
ST18	amygdala	Suppression of tumorigenicity 18 (breast carcinoma) (zinc finger protein)	A1741795	8q11.23	**0.875**	**0.804**	**0.652**	0.711	0.983	0.945
RNASE1	amygdala	"Ribonuclease, RNase A family, 1 (pancreatic)"	AL046791	14q11.2	0.937	**0.932**	**0.760**	0.771	0.962	0.967

Genes associated with suicide.										
Symbol	**Suicide dysregulation**	**Name**	**Best Accession**	**Cytoband**	**Anterior Cingulate Fold Change**		**Amygdala Fold Change**		**Cerebellum Fold Change**	
					Suicide Vs Mood	**Suicide Vs Control**	**Suicide Vs Mood**	**Suicide Vs Control**	**Suicide Vs Mood**	**Suicide Vs Control**
KLK6	amygdala	"Kallikrein 6 (neurosin, zyme)"	CA945202	19q13.3	0.882	**0.878**	**0.739**	0.781	0.930	0.973
ZNF536	amygdala	Zinc finger protein 536	AK091043	19q12			**1.584**	1.603		
CX3CR1	amygdala	Chemokine (C-X3-C motif) receptor 1 (CX3CR1)	CB047889	3p21	0.861	**0.808**	**0.705**	0.779	0.885	0.837
CHL1	cblm	Cell adhesion molecule with homology to L1CAM (close homolog of L1)	BF966608	3p26.1	1.119	1.088	**0.978**	**1.297**	**0.786**	**0.786**
ZBTB16	cblm	Zinc finger and BTB domain containing 16	Z19002	11q23.1	**1.231**	1.235	1.199	**1.161**	**1.446**	**1.625**
FOS	cblm	V-fos FBJ murine osteosarcoma viral oncogene homolog	BM894421	14q24.3	**0.671**	**0.679**	**0.716**	**0.638**	**0.770**	**0.659**
HLA-DPA1	cblm	"Major histocompatibility complex, class II, DP alpha 1"	A1554919	6p21.3	0.874	**0.860**	0.831	**0.839**	**0.771**	**0.799**

TABLE 1B

Symbol	Suicide dys-regulation	Name	Best Ac-cession	Cytoband	Anterior Cingulate Fold Change	
					Suicidal MDD Vs Non-suicidal MDD	Suicidal MDD Vs Control
PRPS2	ancng	Phosphoribosyl pyrophosphate synthetase 2	NM_002765	Xp22.3-p22.2	**1.287**	**1.251**
FGF9	ancng	Fibroblast growth factor 9 (glia-activating factor)	D14838	13q11-q12	**1.270**	**1.288**
RIMS2	ancng	Regulating synap-tic membrane exocytosis 2	BC043144	8q22.3	**1.243**	**1.288**
TM4SF9	ancng	Tetraspanin 5	BX649011	4q23	**1.488**	**1.358**
PCLO	ancng	Piccolo (presy-naptic cytomatrix protein)	AB011131	7q11.23-q21.3	**1.555**	**1.383**
GNB5	ancng	Guanine nucleoti-de binding protein (G protein), beta 5	AK092059	15q21.2	**1.206**	**1.263**
GABRA1	ancng	Gamma-aminobutyric acid (GABA) A recep-tor, alpha 1	NM_000806	5q34-q35	**1.353**	**1.356**
NLK	ancng	Nemo like kinase	BC064663	17q11.2	**1.413**	**1.351**
CDH18	ancng	Cadherin 18, type 2	BC031051	5p15.2-p15.1	**1.217**	**1.260**
NR1D2	ancng	Nuclear receptor subfamily 1, group D, member 2	AB209091	3p24.2	**1.476**	**1.319**

Symbol	Suicide dys-regulation	Name	Best Ac-cession	Cytoband	Anterior Cingulate Fold Change	
					Suicidal MDD Vs Non-suicidal MDD	Suicidal MDD Vs Control
R3HDM	ancng	R3H domain con-taining 1	BX538168	2q21.3	**1.208**	**1.222**
COL5A2	ancng	Collagen, type V, alpha 2	NM_000393	2q 14-q32	**1.209**	**1.226**
	ancng	Cerebellar mRNA	AK126654		**1.229**	**1.303**
NRXN1	ancng	Neurexin 1	AB011150	2p16.3	**1.247**	**1.230**
NCALD	ancng	Neurocalcin delta	AB209015	8q22-q23	**1.284**	**1.306**
CADPS2	ancng	Ca2+-dependentr activator protein for secretion 2	NM_017954		**1.393**	**1.426**
TRIM23	ancng	Tripartite motif-containing 23	NM_001656	5q12.3	**1.231**	**1.227**
STAT4	ancng	Signal transducer and activator of transcription 4	NM_003151	2q32.2-q32.3	**1.228**	**1.314**
HLA-A	ancng	Major histocompa-tibility complex, class I, A	AB209117	6p21.3	**0.771**	**0.788**
LYPDC1	ancng	LY6/PLAUR do-main containing 1	AK122643	2q21.2	**0.819**	**0.787**
RYR1	ancng	Ryanodine recep-tor 1 (skeletal)	NM_000540	19q13.1	**0.749**	**0.791**

TABLE 1C Genes associated with substance abuse comorbidity.

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change	
					Substance Abuse MDD Vs Non-S.A. MDD	Substance Abuse MDD Vs Control
NS3TP1	ancng	HCV NS3-transactivated protein 1	AK000759	2p24.3-q21.3	**1.280**	**1.230**
DZIP1	ancng	DAZ interacting protein 1	NM_198968	13q32.1	**1.259**	**1.247**
STMN4	ancng	Stathmin-like 4	NM_030795	8p21.2	**1.255**	**1.217**
CDH 18	ancng	Cadherin 18, type 2	BC031051	5p15.2-p15.1	**1.269**	**1.238**
KCMF1	ancng	Potassium channel modulatory factor 1	NM_020122	2p11.2	**1.230**	**1.214**
CRYZL1	ancng	Crystallin, zeta (quinone reductase)-like 1	AK057604	21q21.3	**1.461**	**1.341**
OSBPL8	ancng	Oxysterol binding protein-like 8	NM 020841	12q14	**1.551**	**1.403**
FGF14	ancng	Fibroblast growth factor 14	AY188178	13q34	**1.291**	**1.231**
HSPCB	ancng	Heat shock 90kDa protein 1, beta	AY359878	6p12	**1.264**	**1.210**
DDX50	ancng	DEAD (Asp-Glu-Ala-Asp) box polypep-	BC000272	10q22.1	**1.338**	**1.219**

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change	
					Substance Abuse MDD Vs Non-S.A. MDD	Substance Abuse MDD Vs Control
		tide 50				
GNAZ	ancng	G protein, alpha z polypeptide	BC037333	22q11.22	**1.279**	**1.210**
CACNB2	ancng	Calcium channel, voltage-dependent, B2	AB208917	10p12	**1.270**	**1.263**
RASL11B	ancng	RAS-like, family 11, member B	BC025694	4q12	**1.272**	**1.216**
DNM2	ancng	Dynamin 2	AK127033	19p13.2	**0.805**	**0.813**
THG-1	ancng	TSC22 domain family, member 4	NM_030935	7p21-p15	**0.829**	**0.785**

TABLE 2A

Lithium-responsive genes known in literature: confirmed in non-human primates			
Symbol	**UGCluster**	**Cytoband**	**Name**
AP1S1	NM_057089	7q22.1	adaptor-related protein complex 1, sigma 1 subunit
CLOCK	NM_004898	4q12	clock homolog (mouse)
FGF8	NM_033163-5	10q24	fibroblast growth factor 8 (androgen-induced)
GSK3B	NM_002093	3q13.3	glycogen synthase kinase 3 beta
NFKB1	NM_003998	4q24	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)

TABLE 2B

Novel genes that are both lithium-responsive and dysregulated in BPD			
Symbol	**UGCluster**	**Cytoband**	**Name**
A2M	Hs.212838	12p13.3-p12.3	Alpha-2-macroglobulin
AP1S1	Hs.489365	7q22.1	Adaptor-related protein complex 1, sigma 1 subunit
BRD7	In multiple clusters	16q12	bromodomain containing 7
CA10	Hs.463466	17q21	Carbonic anhydrase X
CD74	Hs.436568	5q32	CD74 antigen (invariant polypeptide of major histo-compatibility complex, class II antigen-associated)
CDK5R2	Hs.158460	2q35	Cyclin-dependent kinase 5, regulatory subunit 2 (p39)
CNN2	Hs.169718	21q11.1	Calponin 2
DSTN	Hs.304192	20p12.1	Destrin (actin depolymerizing factor)
ELAVL4	Hs.213050	1p34	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)
FLJ10094	Hs.128258	13q14.11	Hypothetical protein FLJ10094
HNRPH2	Hs.278857	Xq22	Heterogeneous nuclear ribonucleoprotein H2 (H')
IGFBP6	Hs.274313	12q13	Insulin-like growth factor binding protein 6
MOGAT2	Hs.288568	11q13.5	Monoacylglycerol O-acyltransferase 2
MYLK	Hs.477375	3q21	Myosin, light polypeptide kinase
NRIP1	Hs.155017	21q11.2	Nuclear receptor interacting protein 1
PSG5	Hs.558372	19q13.2	Pregnancy specific beta-1-glycoprotein 5
PTP4A2	In multiple clusters	1p35	Protein-tyrosine phosphatase, type 4A, 2P4A2
RPLP2	Hs.437594	11p15.5-p15.4	Ribosomal protein, large, P2
RPS14	Hs.381126	5q31-q33	Ribosomal protein S14
RPS9	In multiple clusters	19q13.4	Ribosomal protein S9

Novel genes that are both lithium-responsive and dysregulated in BPD			
Symbol	**UGCluster**	**Cytoband**	**Name**
SST	Hs.12409	3q28	Somatostatin
UBQLN2	Hs.522668	Xp11.23-p11.1	Ubiquilin 2
UXT	Hs.172791	Xp11.23-p11.22	Ubiquitously-expressed transcript

TABLE 3

Summary of LC dysregulated genes in subjects with major depression disorder			
RefSequ #	**Name**	**p (C vs.MD)**	**FC (fold change) MD vs. C**
A. Glutamate signaling			
NM_004171	SLC1A2 (glial high affinity glutamate transporter)	0.04	-1.26
NM_004172	SLC1A3 (glial high affinity glutamate transporter)	0.03	-1.59
NM_002065	GS (glutamine synthetase)	0.02	-1.28
NM_020346	VGlutT2	0.01	1.31
NM_005271	Glutamate dehydrogenase	0.05	-1.27
NM_181875	mGlutR7 (transcript variant 3)	0.07	-1.06
NM_000827	AMPA1	0.09	1.14
NM_000828	AMPA3	0.01	1.16
NM_175611	GRIK1	0.02	1.22
NM_000838	mGlutR1	0.05	1.08
NM_000842	mGlutR5	0.01	1.16
B. Growth factors and growth related gene			
NM 002006	FGF2	0.10	-1.07
NM_002010	FGF9	0.02	1.16
NM_000142	FGFR3	0.04	-1.17

Summary of LC dysregulated genes in subjects with major depression disorder			
RefSequ #	**Name**	**p (C vs.MD)**	**FC (fold change) MD vs. C**
A. Glutamate signaling			
NM_001007097	TrkB receptor	0.03	-1.45
NM 000163	Growth hormone receptor	0.10	-1.20

C. Glial markers			
NM_002055	Glial fibrillary acidic protein	0.05	-1.32
NM_000165	Gap junction protein, alpha 1, 43kDa (connexin 43)	0.04	-1.86
NM_006783	Gap junction protein, beta 6 (connexin 30)	0.02	-1.93
NM_080388	S100 calcium binding protein A16	0.02	-1.15

TABLE 4

Summary of dysregulated genes in DR of MDD and BPD subjects					
MDD genes					
RefSeq8_ID	**Description**	**FC MD vs. C**	**p value MD vs. C**	**FC BP vs. C**	**p value BP vs. C**
NM_001709	brain-derived neurotrophic factor (BDNF), transcript variant 4, mRNA	-1.207	0.052	-1.164	0.140
NM_003670	basic helix-loop-helix domain containing, class B, 2 (BHLHB2), mRNA	-1.185	0.017	-1.175	0.160
NM_001200	bone morphogenetic protein 2 (BMP2), mRNA	-1.059	0.085	-1.035	0.151
NM_001008540	chemokine (C-X-C motif) receptor 4 (CXCR4), transcript variant 1, mRNA	-1.154	0.001	-1.067	0.260
NM_145793	GDNF family receptor alpha 1 (GFRA1), transcript variant 2, mRNA	-1.062	0.067	-1.020	0.429
NM_013372	gremlin 1, cysteine knot superfamily, (Xenopus laevis) (GREM1), mRNA	-1.153	0.020	-1.099	0.194

Summary of dysregulated genes in DR of MDD and BPD subjects					
MDD genes					
RefSeq8_ID	**Description**	**FC MD vs. C**	**p value MD vs. C**	**FC BP vs. C**	**p value BP vs. C**
NM_002253	kinase insert domain receptor (a type III receptor tyrosine kinase) (KDR), mRNA	-1.081	0.092	-1.065	0.362
NM_005924	mesenchyme homeobox 2 (MEOX2), mRNA	-1.105	0.028	-1.045	0.417
NM_002530	neurotrophic tyrosine kinase, receptor, type 3 (NTRK3), transcript variant 2, mRNA	1.080	0.072	1.090	0.165
NM_002660	phospholipase C, gamma 1 (PLCG1), transcript variant 1, mRNA	1.165	0.098	1.159	0.122
NM_005904	SMAD, mothers against DPP homolog 7 (Drosophila) (SMAD7), mRNA	-1.195	0.056	-1.037	0.439
NM_000346	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal) (SOX9), mRNA	-1.204	0.093	-1.128	0.251
NM_005749	transducer of ERBB2, 1 (TOB1), mRNA	-1.946	0.034	-1.866	0.113
NM_005429	vascular endothelial growth factor C mRNA	-1.150	0.034	-1.099	0.168
NM_000756	corticotropin releasing hormone (CRH), mRNA	1.205	0.021	-1.032	0.146
BPD genes					
RefSeq8_ID	**Description**	**FC MD vs. C**	**p value MD vs. C**	**FC BP vs. C**	**p value BP vs. C**
NM_004281	BCL2-associated athanogene 3 (BAG3), mRNA	-1.207	0.170	-1.345	0.073
NM_203330	CD59 molecule, complement regulatory protein (CD59), transcript variant 1, mRNA	-1.040	0.326	-1.107	0.042

Summary of dysregulated genes in DR of MDD and BPD subjects

MDD genes					
RefSeq8_ID	**Description**	**FC MD vs. C**	**p value MD vs. C**	**FC BP vs. C**	**p value BP vs. C**
NM_000089	collagen, type I, alpha 2 (COL1A2), mRNA	-1.034	0.325	-1.134	0.049
NM_001331	catenin (cadherin-associated protein), delta 1 (CTNND1), mRNA	1.049	0.075	1.083	0.011
NM_001963	epidermal growth factor (beta-urogastrone) (EGF), mRNA	-1.048	0.149	-1.083	0.030
NM_004508	isopentenyl-diphosphate delta isomerase 1 (IDI1), mRNA	-1.168	0.192	-1.246	0.069

RefSeq8 ID	**Description**	**FC MD vs. C**	**p value MD vs. C**	**FC BP vs. C**	**p value BP vs. C**
NM_058004	phosphatidylinositol 4-kinase, catalytic, alpha polypeptide (PIK4CA), transcript variant 2, mRNA	-1.071	0.283	-1.235	0.025
NM 016224	sorting nexin 9 (SNX9), mRNA	-1.105	0.134	-1.162	0.085
NM_003239	transforming growth factor, beta 3 (TGFB3), mRNA	1.022	0.364	1.204	0.026
NM_133646	sterile alpha motif and leucine zipper containing kinase AZK (ZAK), transcript variant 2, mRNA	-1.111	0.159	-1.208	0.094
NM_000898	monoamine oxidase B (MAOB), nuclear gene encoding mitochondrial protein, mRNA	-1.083	0.156	-1.102	0.042
Common genes					
RefSeq8_ID	**Description**	**FC MD vs. C**	**p value MD vs. C**	**FC BP vs. C**	**p value BP vs. C**

RefSeq8 ID	Description	FC MD vs. C	p value MD vs. C	FC BP vs. C	p value BP vs. C
NM_004723	rho/rac guanine nucleotide exchange factor (GEF) 2 (ARHGEF2), mRNA	1.089	0.099	1.073	0.074
NM_078467	cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), transcript variant 2, mRNA	-1.162	0.056	-1.319	0.016
NM_001946	dual specificity phosphatase 6 (DUSP6), transcript variant 1, mRNA	-1.321	0.076	-1.295	0.078
NM_001402	eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), mRNA	-1.416	0.016	-1.419	0.066
NM_032638	GATA binding protein 2 (GATA2), mRNA	1.134	0.040	1.172	0.005
NM_006186	nuclear receptor subfamily 4, group A, member 2 (NR4A2), transcript variant 1, mRNA	-1.869	0.007	-1.877	0.070
NM_002525	nardilysin (N-arginine dibasic conver-tase) (NRD1), mRNA	1.113	0.043	1.141	0.050
NM_002576	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast) (PAK1), mRNA	1.070	0.030	1.085	0.067

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Patentkrav

1. Ligand, der aktiverer FGF-receptor til anvendelse som et antidepressivum i den terapeutiske behandling af et individ med MDD, major depressive disorder, hvor liganden er et NCAM-peptid-mimetikum.

2. Ligand til anvendelse ifølge krav 1, hvor NCAM-peptid-mimetikummet omfatter et FGL-peptid med aminosyresekvensen EVYVVAENQQGKSKA.

3. Ligand til anvendelse ifølge krav 1 eller 2, hvor MDD er forbundet med angst.

4. Ligand til anvendelse ifølge krav 1 eller 2, hvor MDD involverer en vedvarende trist, angst eller tom sindstilstand.

5. Ligand til anvendelse ifølge et hvilket som helst af kravene 1 til 4, hvor individet er et menneske.

6. Ligand til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor liganden administreres med et farmaceutisk acceptabelt bærestof.

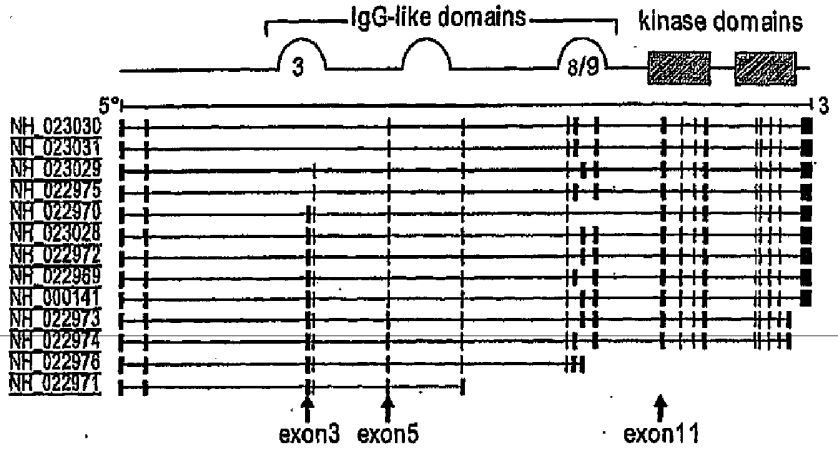
7. Ligand til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor liganden administreres oralt, nasalt, topisk, intravenøst, intraperitonealt eller intrathekalt.

8. Ligand til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor individet er diagnosticeret med MDD.

9. Ligand til anvendelse ifølge et hvilket som helst af kravene 1 til 8, hvor liganden er bundet til en dimeriseringsforbindelse.

10. Ligand til anvendelse ifølge et hvilket som helst af kravene 1 til 9, hvor liganden administreres ved en dosis på ca. 1 ng/kg til 10 mg/kg.

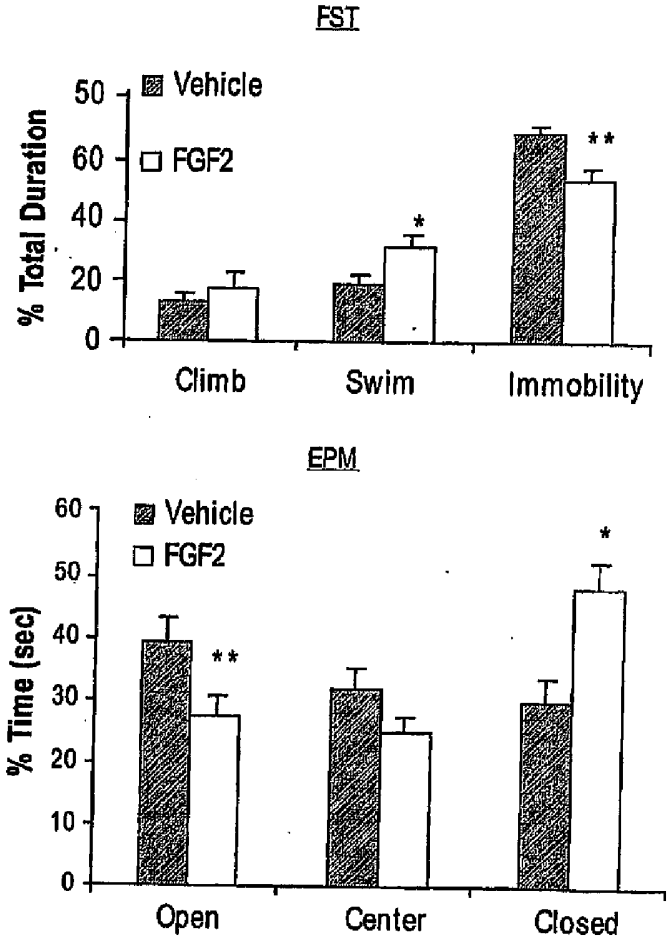
FGFR2 Variant Differences in MDD



brain region	exon5:exon11 ratio (control)	exon5:exon11 ratio (MDD)	p-value
AnCg	1.7	1.5	0.46
DLPFC	1.9	1.3	0.06
HC	2.1	1.4	0.20

FIG. 1

FIG. 2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Climb
Swim
Immobility
EPM
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Open
Center
Closed

FIG. 3

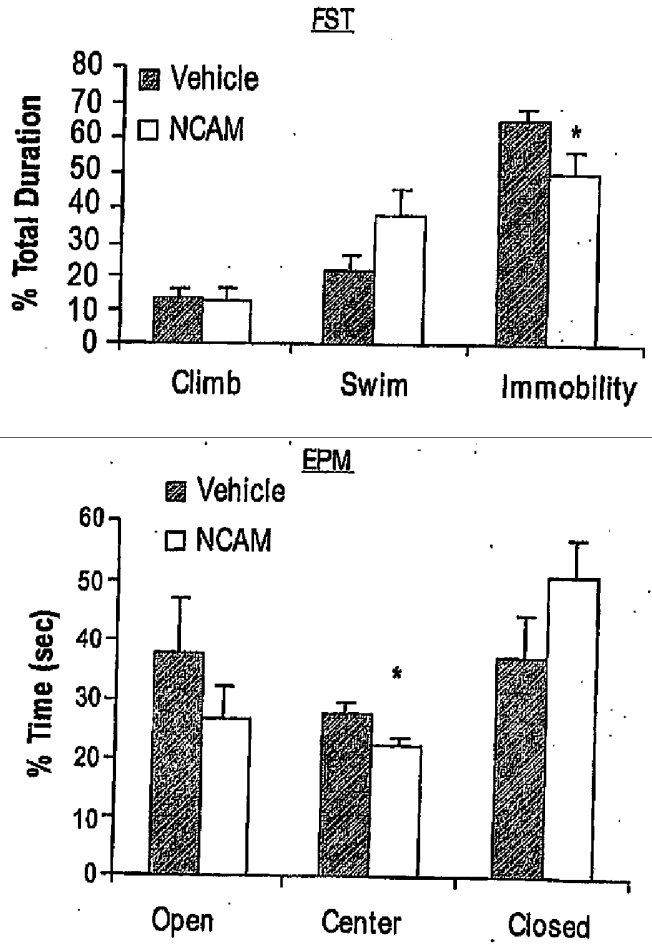
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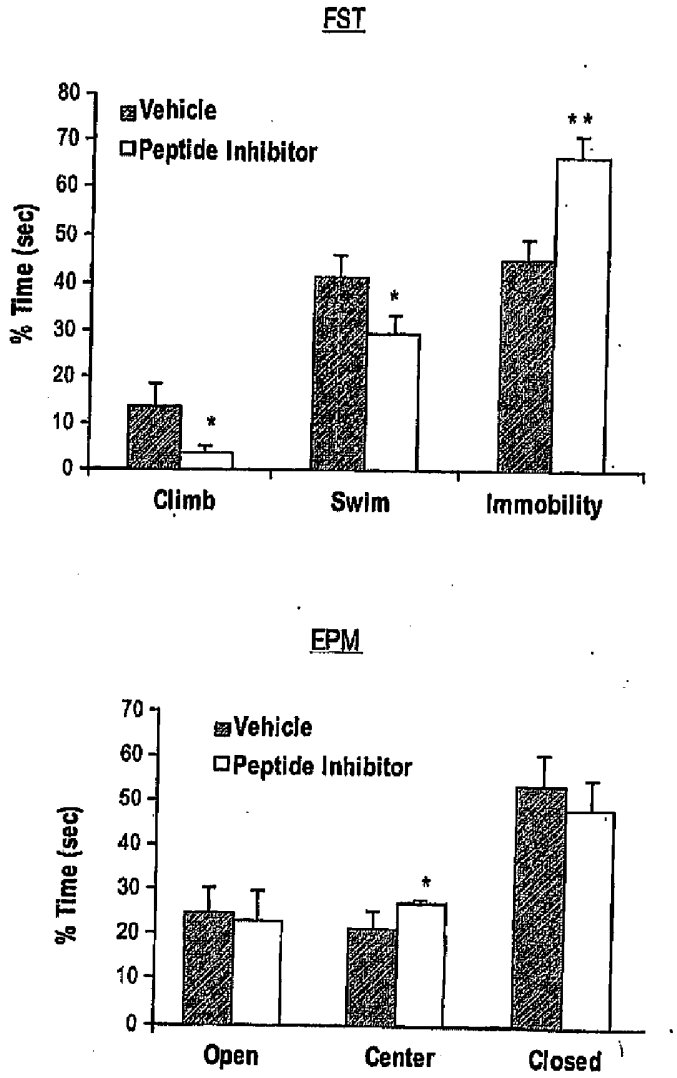
FIG. 4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Dysregulation of genes involved in cAMP signaling pathways in AnCg from patients with BPD

Symbol	Name	UniGene ID	Cytoband	U95Av2	U133A
NPY1R	Neuropeptide Y receptor Y1	Hs.519057	4q31-q32		24.4**
NPY	Neuropeptide Y	Hs.1832	7p15	22.1**	33.0**
SST	Somatostatin	Hs.12409	3q28	29.0**	22.1**
GRM3	Metabotropic Glutamate receptor 3	Hs.112621	7q21	33.4**	34.4**
EDG2	Endothelial differentiation GPCR 2	Hs.126667	9q31.3	24.1*	18.2*
GNAI1	G protein alpha inhibiting activity 1	Hs.134587	7q21	40.4**	
PDE1A	Phosphodiesterase 1A	Hs.416061	2q32	20.1**	24.1**
PDE8A	Phosphodiesterase 8A	Hs.9333	15q25	16.3*	12.5*
PKIA	Protein kinase A inhibitor alpha	Hs.433700	8q21	14.2*	16.6*
CDK5	Cyclin-dependent kinase 5	Hs.166071	7q36	10.5*	12.0*
PPP1CA	Protein phosphatase 1, catalytic alpha	Hs.183994	11q13	13.1*	16.8*

***The p-values showing a false discovery rate (FDR) of less than 5%*
** p-value of less than 0.05 regardless of FDR*

The probability that the group of molecules that act negatively on cAMP signaling activity are detected as genes increased in BPD by chance, based on a hypergeometric distribution, was p = 0.037 (q = 0.36).

FIG. 5

FIG. 6

Dysregulation of genes involved in cAMP signaling pathways in AnCg from patients with MDD

				MDD Cohort A		MDD Cohort B
Symbol	Name	UniGene ID	Cytoband	U95Av2	U133A	U133A
PDE8A	Phosphodiesterase 8A	Hs.9333	15q25	-24.3**	-23.1**	-17.8*
RGS20	Regulator of G-protein signalling 20	Hs.368733	8q12	-28.2**	-29.2**	-13.9*
EDG1	Endothelial differentiation GPCR 1	Hs.154210	1p21	-19.7*	-21.2**	-10.4*
PPP1R3C	Protein phosphatase 1, regulatory 3C	Hs.303090	10q23-q24	-35.4**	-63.2**	-21.4**

***The p-values showing a false discovery rate (FDR) of less than 5%*
** p-value of less than 0.05 regardless of FDR*

FIG. 7

Dysregulation of genes involved in phosphatidylinositol signalling pathways in AnCg from patients with BPD

Symbol	Name	UniGene ID	Cytoband	U95Av2	U133A
EDG2	Endothelial differentiation GPCR 2	Hs.126667	9q31.3	24.1*	18.2*
ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B	Hs.528087	1q41-q43	14.8*	11.5*
INPP1	Inositol polyphosphate-1-phosphatase	Hs.32309	2q32	21.5**	24.9**
CDS1	CDP-diacylglycerol synthase 1	Hs.444924	4q21.23	-29.5**	-24.2*
PIK3C2A	Phosphoinositide-3-kinase catalytic 2A	Hs.175343	11p15-p14		22.3**
PIK3C2B	Phosphoinositide-3-kinase catalytic 2B	Hs.497487	1q32	16.6*	14.8*
PIK3R1	Phosphoinositide-3-kinase regulatory 1	Hs.132225	5q13	-32.0**	
PRKCI	Protein kinase C_ (iota)	Hs.478199	3p25-q27	-29.5**	

***The p-values showing a false discovery rate (FDR) of less than 5%*
** p-value of less than 0.05 regardless of FDR*

The probability that the group of the phosphatidylinositol 3-kinases are detected by chance as differentially expressed genes in the comparison between BPD and control, based on a hypergeometric distribution, was p = 0.042 (q = 0.36).

FIG. 8

Dysregulation of genes involved in phosphatidylinositol signalling pathways in AnCg from patients with MDD

				MDD Cohort A		MDD Cohort B
Symbol	Name	UniGene ID	Cytoband	U95Av2	U133A	U133A
NTSR2	Neurotensin receptor 2	Hs.131138	2p25.1	-13.9*	-18.6*	-24.7**
EDNRB	Endothelin receptor type B	Hs.82002	13q22.		-31.2**	-15.9*
ITPKB	Inositol 1,4,5-trisphophate 3-kinase B	Hs.528087	1q41-q43	-12.7*	-12.3*	-34.0**
INPP5F	Inositol polyphosphate-5-phosphatase F	Hs.369755	10q26		21.8**	
ITPR1	Inositol 1,4,5-triphosphate receptor 1	Hs.374613	3p26-p25	13.0*	17.4*	
PRKCB1	Protein kinase C beta 1	Hs.460355	16p11	14.8*	11.5*	
PIK3C2A	Phophoinositide-3-kinase catalytic 2A	Hs.175343	11p15-p14	-14.1*	-35.9**	-20.2*

***The p-values showing a false discovery rate (FDR) of less than 5%*
** p-value of less than 0.05 regardless of FDR*

FIG. 9

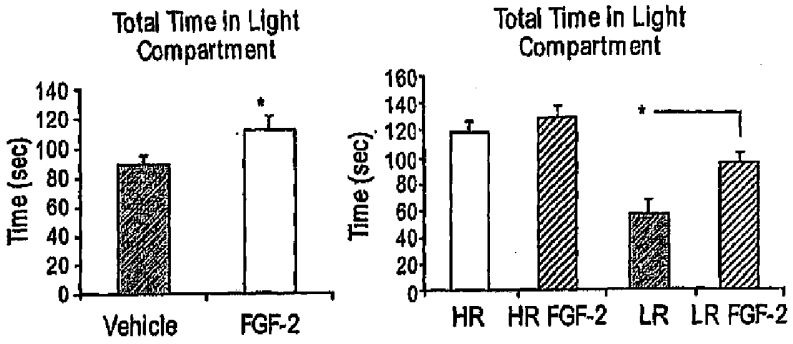
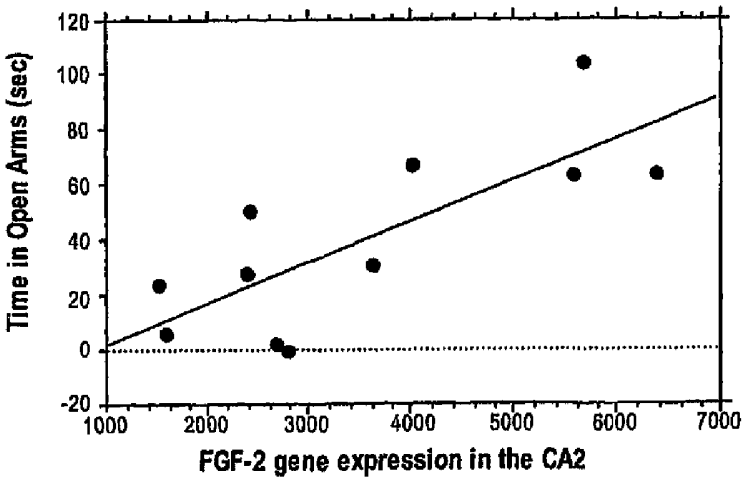
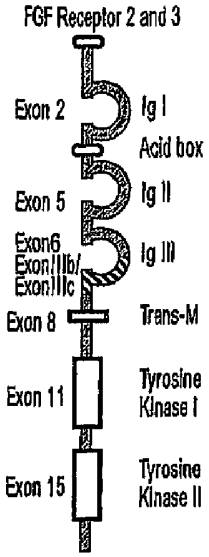


FIG. 10



Correlation Coefficient= .77; P<003.

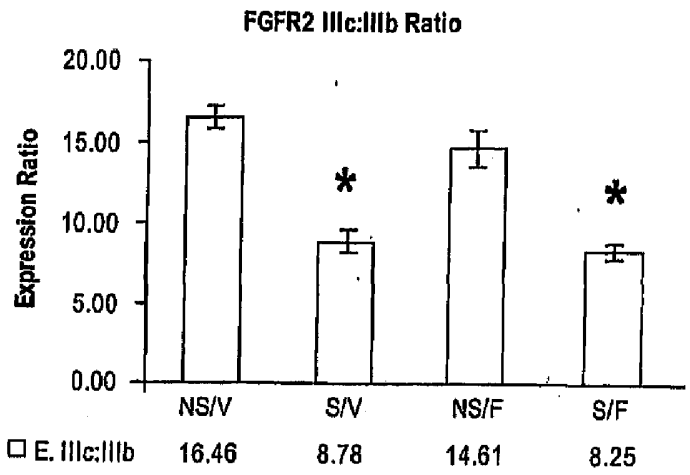
FIG. 11

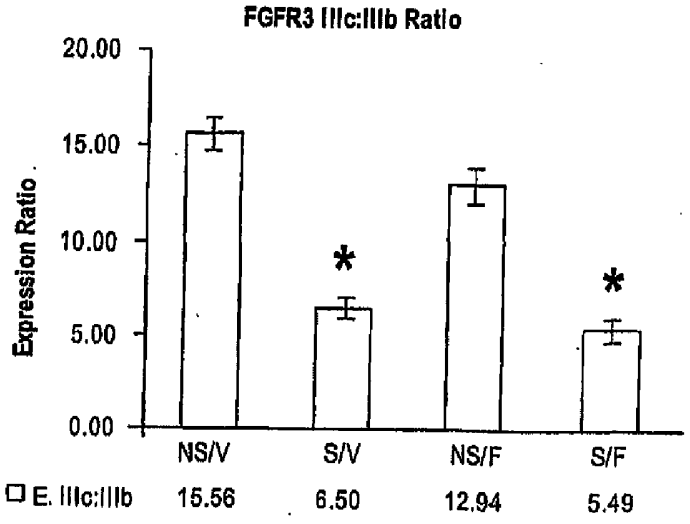


FGFR2 and FGFR3 Primer Sequences

Amplicon	Primer	Sequence	Primer	Sequence
Exon 2	R2 F	5'-GCCGTGATCAGTTGGACTAAG-3'	R3 F	5'-AGAGGCTTCAAGTGCTAAACG-3'
	R2 R	5'-TGTGGCACCTTTTATCTGGAG-3'	R3 R	5'-GCACACTAAAGTGGCACAGC-3'
Exon 5	R2 F	5'-TATGGAAAGTGTGGTCCCATC-3'	R3 F	5'-TGGAGCTTGGTCATGGAAAG-3'
	R2 R	5'-ACATCAAGGTGGTAGGTGTGG-3'	R3 R	5'-GGATGCTGCCAAACTTGTTC-3'
Exon 6	R2 F	5'-GGAGGGGACGTAGAATTTGTC-3'	R3 F	5'-CCAACCAGACAGCCGTTC-3'
	R2 R	5'-CTTCAGGACCTTGAGGTAGGG-3'	R3 R	5'-CATTCACCTCCACGTGCTT-3'
Exon IIIb	R2 F	5'-GGGGATAAATAGCTCCAATGC-3'	R3 F	5'-CCTGGATCAGTGAGAATGTGG-3'
	R2 R	5'-CATATATATTCCCCAGCATCCATC-3'	R3 R	5'-AAATTGGTGGCTCGACAGAG-3'
Exon IIIc	R2 F	5'-ACACCACGGACAAAGAAATTG-3'	R3 F	5'-TGTCCTTGCACAATGTCACC-3'
	R2 R	5'-ATAGAATTACCCGCCAAGCAC-3'	R3 R	5'-ACGCAGAGTGATGGGAAAAC-3'
Exon 8	R2 F	5'-GATCACAGCTTCCCCAGATTAC-3'	R3 F	5'-GGAGGAGCTGATGGAAGTTG-3'
	R2 R	5'-TCTTGGTCGTGGTCTTCATTC-3'	R3 R	5'-CCACCAGGATGAAGAGGAAG-3'
Exon 11	R2 F	5'-AGAGAAGGACCTGTCTGACCTG-3'	R3 F	5'-ATGCCACTGACAAGGACCTG-3'
	R2 R	5'-CCCAGGAGGTTGATGATGTTC-3'	R3 R	5'-CCCCCAACAGGTTAATGATG-3'
Exon 15	R2 F	5'-GTCCTTCGGGGTGTTAATGTG-3'	R3 F	5'-TCCTTTGGTGTCCTCCTCTG-3'
	R2 R	5'-AGTTCATTGGTGCAGTTGGTG-3'	R3 R	5'-CAGTTGGCTGGCTTGTCC-3'

FIG. 12


FGFR2 IIIc:IIIb Ratio
Expression Ratio
20.00
15.00
10.00
5.00
0.00
*
*
NS/V
S/V
NS/F
S/F
E. IIIc:IIIb
16.46
8.78
14.61
8.25


FGFR3 IIIc:IIIb Ratio
Expression Ratio
20.00
15.00
10.00
5.00
0.00
*
*
NS/V
S/V
NS/F
S/F
E. IIIc:IIIb
15.56
6.50
12.94
5.49