(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 May 2009 (07.05.2009)

WIPO | PCT

(10) International Publication Number
WO 2009/059316 A9

(51) International Patent Classification:
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2008/082279

(22) International Filing Date:
3 November 2008 (03.11.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/985,146 2 November 2007 (02.11.2007) US

(71) Applicant *(for all designated States except US)*: **THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY** [US/US]; 1705 El Camino Real, Palo Alto, CA 94306-1106 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **AKIL, Huda** [US/US]; 3351 Windshadow Drive, Ann Arbor, MI 48105 (US). **WATSON, Stanley** [US/US]; 3351 Windshadow Drive, Ann Arbor, MI 48105 (US). **TURNER, Cortney** [US/US]; 4816 Washtenaw Avenue, Apt. B3, Ann Arbor, MI 48108 (US).

[Continued on next page]

(54) Title: FGF9-RELATED METHODS FOR TREATING ANXIETY

(57) Abstract: The present application relates to the treatment of anxiety in a subject. The invention provides novel diagnostic markers and assays, as well as agents and compounds useful for treating patients who suffer from mental illnesses and anxiety disorders.

Fig. 13 (Sheet 1)

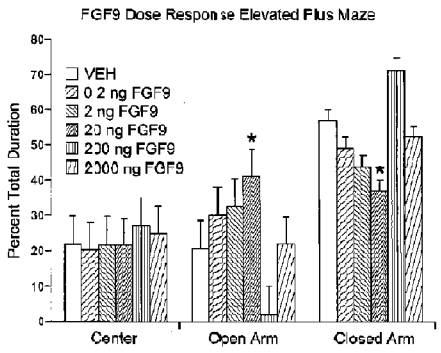
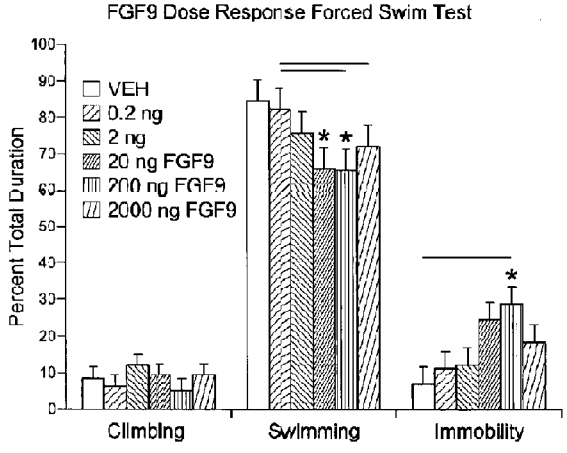


Fig. 13 (Sheet 2)



WO 2009/059316 A9

(74) **Agents**: **PARENT, Annette, S.** et al.; Townsend and Townsend and Crew LLP, Two Embarcadero Center, Eighth Floor, San Francisco, CA 94111-3834 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(48) **Date of publication of this corrected version**:
19 March 2015

(15) **Information about Correction**:
see Notice of 19 March 2015

FGF9-RELATED METHODS FOR TREATING ANXIETY

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/985,146, filed November 2, 2007, the teachings of which are incorporated herein by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] 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)).

[0003] Anxiety is a normal reaction to stress. It helps one deal with a tense situations, prepare and take exams, or stay focused on an important speech. In general, it helps one cope. When anxiety becomes an excessive, irrational dread of everyday situations, however, it can be disabling. Examples of anxiety disorders include: generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, post-traumatic stress disorder and social phobia (or social anxiety disorder). Anxiety may also be associated with mental disorders such as those described above, including major depression disorder.

[0004] Anxiety disorders are considered the most prevalent of psychiatric disorders, 22% of the total population across the seven major pharmaceutical markets suffering from an anxiety disorder, causing considerable individual burden and imposing substantial social burdens, costing the economy over $42 billion in the US alone. However, the exact prevalence of such disorders is difficult to quantify due to the lack of epidemiological data, plus the high incidence of comorbidity with other psychiatric disorders. Furthermore, physicians estimate that only 26% of the population who suffer from an anxiety disorder are diagnosed and therefore treated. This is a result of combination of poor knowledge of the disorder by both patients and physicians.

[0005] With approximately 30% of patients requiring second-line therapy, current international guidelines, such as those published by the World Federation of Societies of Biological Psychiatry, recommend the use of selective serotonin re-uptake inhibitors ("SSRIs") as first-line therapy for the treatment of anxiety. Nonetheless, despite the widespread use of SSRIs, approximately 30% of patients require second-line therapy. This suggests that current therapies are ineffective in up to a third of anxiety disorder patients tehrefore there is a significant need for anxiety drugs with improved efficacy.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides novel assays for the diagnosis and detection of various mental illnesses. The assays, which include assays for detecting the level of expression of various genes associated with mental disorders, allow the practitioner to obtain a more accurate diagnosis of mental illness in a subject, and allow the practitioner to distinguish between various mental illnesses and associated pathologies. The invention also provides compositions useful for practicing the methods of invention, for developing further diagnostic methodologies, and new therapeutics to aid in the treatment of mental illness, particularly anxiety.

[0007] In one embodiment, the present invention provides methods of correlating the expression of FGFR2 splice variants with MDD. Because of the relationship between MDD, BP and psychotic disorders such as schizoaffective disorders or psychotic depression, the splice variants described herein are unique to MDD and can be used for differential diagnosis, treatment and prevention of MDD.

[0008] In another embodiment, the present invention provides methods for altering the behavioral profiles of rats by injecting the rats with FGF2, an NCAM peptide mimetic, and a peptide inhibitor of FGF receptors. 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. In one embodiment of the invention, ligands that activate FGF receptors are used for their antidepressant effects in the therapeutic treatment of individuals with MDD.

[0009] In another embodiment, the invention provides a set of genes associated with suicide in the amygdala and methods for correlating expression of one or more of those genes with suicidal tendencies (Table 1A and Table 1B). In a related embodiment, the invention

provides a set of genes associated with suicide, co-morbidity with substance abuse in MDD patients (Table 1C) and methods of detecting one or more of those genes, correlating those genes with suicide risk in appropriate patients, and methods of treating individuals identified as suicidal or likely to become suicidal.

[0010] In another embodiment, the invention also provides methods of correlating the differential expression of particular lithium-responsive genes (Table 2A and Table 2B**)** with bipolar affective disorder.

[0011] In yet other embodiment, the invention provides methods for increasing the memory and learning abilities of adult animals by treating early postnatal animals with FGF2. In yet another embodiment, the invention provides methods for treating memory and learning disabilities in animals deficient in active FGF2 by treating early postnatal animals with FGF2.

[0012] In another embodiment, the invention provides a method for facilitating the diagnosis of a mood disorder in a subject, comprising the steps of: (i) measuring the level of expression of a gene, wherein the gene is selected from the group consisting of the genes listed in Figure 5, Figure 6, Figure 7, Figure 8, Table1, Table 2B, Table 3, and/or Table 4; (ii) determining whether the gene is dysregulated relative to a control, wherein dysregulation of the gene indicates an increased likelihood that the subject suffers from a mood disorder; and (iii) recording or reporting any finding with respect to the increased likelihood, *i.e.*, reporting whether there is or is not an increased likelihood that the subject suffers from a mood disorder.

[0013] In a related embodiment, the mood disorder in question is bipolar disorder, and the gene whose dysregulation is analyzed is selected from the group consisting of the genes listed in Figure 5, Figure 7, Figure 8, Table 2B, and/or Table 4. In another related embodiment, the mood disorder is major depression disorder and the gene is selected from the group consisting of the genes listed in Figure 6, Figure 8, Table 1 and/or Table 3.

[0014] In yet another related embodiment of the method for facilitating the diagnosis of a mood disorder in a subject, the gene dysregulation, which is detected and measured, occurs in the subject's brain. In yet another related embodiment, the brain tissue in which the dysregulation occurs is selected from the group consisting of the locus coeruleus, the dorsal raphe, the anterior cingulate cortex, the dorsolateral prefrontal cortex, the hippocampus, and the amygdala. In yet another related embodiment, the gene dyrsegulation is detected in a cell

in which the observed gene expression reflects the gene expression observed in the brain, *e.g.*, a lymphocyte cell.

[0015] In yet another related embodiment of the method for facilitating the diagnosis of a mood disorder in a subject, the dysregulation of gene expression is assayed by detecting messenger RNA transcribed from the gene or genes of interest. In yet another related embodiment, gene expression is assayed by selectively detecting, directly or indirectly, the protein product of the gene or gene of interest. In yet another related embodiment, detecting messenger RNA transcribed from the gene of interest comprises the steps of (i) contacting said mRNA with a reagent which selectively associates with said messenger RNA; and (ii) detecting the level of said reagent which selectively associates with said mRNA.

[0016] In another related embodiment of the method for facilitating the diagnosis of mood disorder in a subject, the measured level of expression of the gene of interest is higher than a level associated with humans without a mood disorder. In yet another related embodiment, the level of expression of the gene is lower than a level associated with humans without a mood disorder, *i.e.*, the gene is downregulated in subjects with a mood disorder.

[0017] In another related embodiment of the method for facilitating the diagnosis of mood disorder in a subject, the level of expression of the gene is detected using a microarray assay, and wherein said gene is one of at least two genes on the microarray.

[0018] In other embodiments of the method for facilitating the diagnosis of mood disorder in a subject, the gene is selected from Table 1 and the mood disorder is suicidal. In a related embodiment, the subject was previously diagnosed with a mood disorder associated with an increased likelihood of suicidal activity. In yet another related embodiment, the subject was previously diagnosed with a mood disorder selected from the group consisting of major depression, bipolar disorder, and schizophrenia. In yet another related embodiment, the method further comprises prescribing a treatment for the subject which reduces the likelihood of a suicide attempt by the subject.

[0019] In other embodiments of the method for facilitating the diagnosis of mood disorder in a subject, the subject has symptoms of both bipolar disorder and major depressive disorder, and the gene of interest is differently expressed in bipolar subjects versus major depression disorder subjects, thereby facilitating a diagnosis of bipolar disorder or major depressive disorder in said subject.

[0020] In other embodiments of the method for facilitating the diagnosis of mood disorder in a subject, the gene of interest is dysregulated in substance-abusing MDD subjects versus MDD subjects who are not substance abusers, and the gene of interest is selected from the dysregulated genes listed in Table 1C, thereby facilitating a diagnosis of MDD versus a diagnosis of MDD in addition to substance abuse.

[0021] In another embodiment, the invention provides a method of identifying a compound for treatment or prevention of a mood disorder, the method comprising the steps of: (i) contacting the compound with a polypeptide or polynucleotide corresponding to a dysregulated gene selected from the group of dysregulated genes listed in Figure 5, Figure 6, Figure 7, Figure 8, Table1, Table 2B, Table 3, and Table 4; and (ii) determining the functional effect of the compound upon the polypeptide or polynucleotide (*e.g.*, inhibition or enhancement of activity), thereby identifying a compound for treatment or prevention of a mood disorder. In a related embodiment, the contacting step is performed in vitro. In yet another related embodiment, the polypeptide is expressed in a cell and the cell is contacted with the compound. In yet another related embodiment, the mood disorder is selected from the group consisting of bipolar disorder, major depression disorder, suicidal, and substance abuse comorbidity. In yet another related embodiment, the method further comprising administering the identified or candidate compound to an animal and determining the effect on the animal, *e.g.*, determining the effect on the animal's mental health and behavioral phenotype.

[0022] In another embodiment, the invention also provides a method of treating a subject who is prone to suicide, comprising the step of administering to the subject a therapeutically effective amount of a polypeptide, the polypeptide encoded by a polynucleotide corresponding to a gene listed in Table 1 or Table 2.

[0023] In another embodiment, the invention also provides a method of treating symptoms of anxiety in a subject (*e.g.,* an animal such as a mouse, cat, dog, horse or human), comprising the step of administering a sufficient amount of FGF2 peptide to the subject after the subject has been diagnosed with anxiety or an illness associated with anxiety. In a related embodiment, the subject is a human. In another related embodiment, the sufficient amount of FGF2 is a dose administered at least twice weekly over a period at least one week in length. In yet another related embodiment, the illness being treated is Major Depression or Major Depressive Disorder.

[0024] In another embodiment, the invention provides a method for diagnosing a human suffering from chronic stress comprising a) obtaining a nucleic acid sample from the subject; and b) determining the exon IIIb:IIIc splice variant ratio of the expressed gene selected from the group consisting of FGFR2 and FGFR3, wherein a ratio less than approximately 10 is associated with an increased likelihood that said human is suffering from chronic stress. In a related embodiment, the gene is FGFR2. In another related embodiment, the gene is FGFR3. In another related embodiment, the method further comprises administering a pharmacological treatment to a human diagnosed with chronic stress using the method.

[0025] In another embodiment, the invention provides a method for identifying a compound which alters the exon IIIb:IIIc splice variant ratio of an expressed gene selected from the group consisting of FGFR2 and FGFR3 in a living animal, comprising a) identifying at least one animal suffering from chronic stress; b) measuring the exon IIIb:IIIc splice variant ratio in said at least one animal; c) administering a test compound to said at least one animal; d) measuring the splice variant ratio a second time after the administration of said test compound; recording the identity of the test compound if said measurement shows that the splice variant ratio is increased.

[0026] In another embodiment, the invention provides a method for treating a subject suffering from a glutamatergic imbalance comprising administering to the subject a sufficient amount of a compound which targets a molecule selected from the group consisting of: glial transporters, glutamine synthetase, AMPA, kainate, GRM1 and GRM7.

[0027] In yet another embodiment, the invention provides a method for increasing neurite growth in a subject suffering from MDD, comprising administering to the subject a sufficient amount of a compound which targets FGFR3, TrkB receptor, or a growth hormone receptor, or which mimics the actions of FGF2.

[0028] In another embodiment, the invention provides a method for detecting global glial alterations in a subject suffering from MDD, comprising the steps of determining the level of gene expression in the LC region of a subject, wherein at least one gene whose level is examined is a glial marker gene selected from the group of glial marker genes in Table 3.

[0029] In yet another embodiment, the invention provides a method for distinguishing between BP and MDD in a human subject, comprising a) measuring the level of expression of at least one MDD- or BP-specific gene in DR tissue of said subject, wherein said MDD- or

BP-specific gene is selected from the MDD- or BP-specific dysregulated genes listed in Table 4; b) and identifying an increased likelihood that said subject suffers from BP versus MDD, wherein downregulation of an MDD-specific gene in Table 4 correlates with an increased likelihood of MDD in said subject, and wherein downregulation of a BP-specific gene in Table 4 correlates with an increased likelihood of BP in said subject. In a related embodiment, the method further comprises recording or reporting the risk of developing BP or MDD. In a related embodiment, the risk is reported to a physician or to the subject.

[0030] In yet another embodiment, the invention provides a method for identifying a human subject with an increased risk of BP or MDD, comprising: (i) measuring the level of expression of a dysregulated gene selected from the genes listed in Table 3; and (ii) correlating said measurement with an increased risk of BP or MDD in said subject. In a related embodiment, the method further comprises recording or reporting the risk of developing BP or MDD. In a related embodiment, the risk is reported to a physician or to the subject.

[0031] In yet another embodiment, the invention provides a method for facilitating the diagnosis of major depression disorder in a subject, comprising the steps of: (i) measuring the ratio of expression of FGFR2 exon 5 to FGFR2 exon 11; (ii) determining whether said ratio is lower than a control, wherein a lower ratio indicates an increased likelihood that said subject suffers from major depression disorder; and (iii) recording or reporting any finding with respect to said increased likelihood. In a related embodiment, the expression ratio is the ration in said subject's dorsolatereral prefrontal cortex.

[0032] In another embodiment, the invention provides a method for facilitating the diagnosis of major depression disorder in a subject, comprising the steps of: (i) measuring the expression of FGFR2 exon 9; (ii) determining whether said expression is lower than a control, wherein a lower level of expression indicates an increased likelihood that said subject suffers from major depression disorder; and (iii) recording or reporting any finding with respect to said increased likelihood. In a related embodiment, the expression is in said subject's dorsolatereral prefrontal cortex.

[0033] In another embodiment, the invention provides a method for improving memory in an animal, comprising administering FGF2 to said animal. In a related embodiment, the animal is a rodent, cat, dog, horse, primate or human. In yet another related embodiment,

administration occurs within 48 hours of the birth of said animal. In another related embodiment, the FGF2 is administered subcutaneously. In yet another related embodiment, the FGF2 is administered at 20 ng/g body weight. In yet another related embodiment, the invention provides a non-human animal, *e.g.,* a rodent, which has been treated with FGF2. In a related embodiment, the animal is an adult animal, previously treated with FGF2, with an improved memory relative to an adult animal which was not previously treated (*e.g.*, treated shortly after birth). In other related embodiments, the expression of NCAM in the FGF2-treated animal is decreased relative to that observed in similar untreated animals. In other related embodiments, the expression of at least one gene selected from the group consisting of GAP-43, Rgs4, trkB, CCK, SST and Vgf is increased in the FGF2-treated animals relative to an untreated animal.

[0034] In yet another embodiment, the invention provides a method for alleviating one or more symptoms of anxiety in a mammalian subject, comprising administering an effective amount of FGF9 to the subject. In a related embodiment, the effective amount of FGF9 may be administered to the subject by injection. In another related embodiment, the subject is diagnosed with an anxiety disorder before treatment. In yet another related embodiment, the subject has been previously diagnosed with major depression disorder or a similar disorder with anxiety-related symptoms. In still another embodiment, the effective amount of FGF9 is acutely administered to the subject, for example, when the symptoms of anxiety are deemed severe enough to warrant administration. In a further embodiment, the subject is a human.

[0035] In yet another embodiment, the invention provides a method for reducing risk-taking behavior in a mammalian subject, comprising administering an effective amount of FGF9 to said subject. In a related embodiment, the effective amount of FGF9 may be administered to the subject by injection. In another related embodiment, the subject has high FGF2 levels, is prone to risk-taking behavior, or a combination thereof. In yet another related embodiment, the subject has been previously diagnosed with one or more symptoms associated with risk-taking behavior. In still another embodiment, the effective amount of FGF9 is chronically administered to the subject. In a further embodiment, the subject is a human.

[0036] As described herein, the present invention relies in part on the surprising discovery that (1) acute administration of FGF2 decreases depression-like behavior and produces an anxiogenic effect, whereas chronic FGF2 administration decreases depression-like behavior

and produces an anxiolytic effect; and (2) acute administration of FGF9 produces an anxiolytic effect and increases depression-like behavior, whereas chronic FGF9 administration produces an anxiogenic effect and increases depression-like behavior. Without being bound to any particular theory, these findings indicate that FGF9 may modulate and/or compete with the effects of FGF2 and that FGF9 may be a physiological antagonist of FGF2. As a non-limiting example, FGF9 may counterbalance the effects of FGF2 in patients with high FGF2 levels and reduce risk-taking behavior.

[0037] In yet another embodiment, the invention provides a method for administering an effective amount of an siRNA that silences FGF9 gene expression to a mammalian subject. In certain instances, the siRNA may be used for treating anxiogenic disorders. In certain other instances, the siRNA may be used for treating anxiolytic disorders. In further instances, the siRNA may be used for treating mood disorders or psychotic disorders. In a related embodiment, the effective amount of the siRNA may be administered to the subject using a carrier system. In another related embodiment, the effective amount of the siRNA may be administered to the subject by injection. In a further embodiment, the subject is a human.

[0038] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] 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.

[0040] 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.

[0041] 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, respectively.

[0042] 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, respectively.

[0043] FIGURE 5 shows a table listing genes in the cAMP signaling pathway whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) from patients with bipolar disorder (BPD).

[0044] FIGURE 6 shows a table listing genes in cAMP signaling pathways whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) of patients with major depression disorder (MDD).

[0045] FIGURE 7 shows a table listing genes in the phosphatidylinositol signaling pathway whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) of patients with bipolar disorder (BPD).

[0046] FIGURE 8 shows a table listing genes in the phosphatidylinositol signaling pathway whose expression is significantly dysregulated in the anterior cingulate cortex (AnCg) of patients with major depression disorder (MDD).

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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).

[0051] FIGURE 13 shows that acute administration of FGF9 (20 ng, i.c.v.) had an anxiolytic effect in rats, as measured by the duration of time spend in the closed arm portion of the test maze versus the open arm portion.

[0052] FIGURE 14 shows that chronic administration of FGF9 (20 ng, i.c.v.) daily for 3 weeks increased the depression-like effect seen in the forced swim test compared to acute administration. Chronic FGF9 had an anxiogenic effect on the elevated plus maze task, which is opposite to the anxiolytic effect seen with acute administration.

DEFINITIONS

[0053] 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.

[0054] 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).

[0055] "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.

[0056] "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.

[0057] "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.

[0058] "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.

[0059] 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.

[0060] An "agonist" refers to an agent that binds to a polypeptide or polynucleotide of the invention, stimulates, increases, activates, facilitates, enhances activation, sensitizes or up regulates the activity or expression of a polypeptide or polynucleotide of the invention.

[0061] An "antagonist" refers to an agent that inhibits expression of a polypeptide or polynucleotide of the invention or binds to, partially or totally blocks stimulation, decreases, prevents, delays activation, inactivates, desensitizes, or down regulates the activity of a polypeptide or polynucleotide of the invention.

[0062] "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 of the invention 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 of the invention, *e.g.*, antagonists. Activators are agents that, *e.g.*, induce or activate the expression of a polypeptide or polynucleotide of the invention or bind to, stimulate, increase, open, activate, facilitate, enhance activation or enzymatic activity, sensitize or up regulate the activity of a polypeptide or polynucleotide of the invention, *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 of the invention and then determining the functional effects on a polypeptide or polynucleotide of the invention activity. Samples or assays comprising a polypeptide or polynucleotide of the invention 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 of the invention relative to the control is about 80%, optionally 50% or 25-1%. Activation is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is 110%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

[0063] 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.

[0064] A "small organic molecule" refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 Daltons and less than about 2500 Daltons, preferably less than about 2000 Daltons, preferably between about 100 to about 1000 Daltons, more preferably between about 200 to about 500 Daltons.

[0065] An "siRNA" or "RNAi" refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the siRNA expressed in the same cell as the gene or target gene. "siRNA" or "RNAi" thus refers to the double stranded RNA formed by the complementary strands. The complementary portions of the siRNA that hybridize to form the double stranded molecule typically have substantial or complete identity. In one embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA. Typically, the siRNA is at least about 15-50 nucleotides in length (*e.g.*,

each complementary sequence of the double stranded siRNA is 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, preferable about preferably about 20-30 base nucleotides, preferably about 20-25 or about 24-29 nucleotides in length, *e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[0066] 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.

[0067] "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 of the invention (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 of the invention or depletion of an substrate; measurement of changes in protein levels of a polypeptide of the invention; 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.

[0068] 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.

[0069] "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.

[0070] "Antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0071] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0072] Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (*see*, Paul (Ed.) *Fundamental Immunology*, Third Edition, Raven Press, NY (1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA

methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies (*e.g.*, single chain Fv).

[0073] 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 of the invention. 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), which are incorporated herein by reference). 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 within the scope of the invention if it is capable of carrying out the binding or enzymatic activities of a polypeptide or polynucleotide of the invention or inhibiting or increasing the enzymatic activity or expression of a polypeptide or polynucleotide of the invention.

[0074] 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).

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] "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.

[0081] 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 of the invention.

[0082] 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)).

[0083] "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.

[0084] 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.

[0085] 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.

[0086] 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)).

[0087] 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.

[0088] 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.

[0089] 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.

[0090] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (*e.g.*, total cellular or library DNA or RNA).

[0091] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60° C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5X SSC, and 1% SDS, incubating at 42°C, or 5X SSC, 1% SDS, incubating at 65°C, with wash in 0.2X SSC, and 0.1% SDS at 65°C. Such washes can be performed for 5, 15, 30, 60, 120, or more minutes. Nucleic acids that hybridize to the genes listed in FIGURE 1, FIGURES 5-8, or Tables 1-4 are encompassed by the invention. Also encompassed by the invention are arrays comprising nucleotides for detecting the expression of two or more of the genes listed in FIGURE 1, FIGURES 5-8, or Tables 1-4.

[0092] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. Such washes can be performed for 5, 15, 30, 60, 120, or more minutes. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

[0093] For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C depending on primer length. For high stringency PCR amplification, a temperature of about 62°C is typical, although high stringency annealing temperatures can range from about 50°C to about 65°C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90°C - 95°C for 30 sec - 2 min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72°C for 1 - 2 min. Protocols and guidelines for low and high stringency amplification reactions are provided, *e.g.*, in Innis *et al.*, *PCR Protocols, A Guide to Methods and Applications* (1990).

[0094] 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.

[0095] 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 protein 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.

[0096] 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).

[0097] 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.

[0098] The phrase "specifically (or selectively) binds to an antibody" or "specifically (or selectively) immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised against a protein having an amino acid sequence encoded by any of the polynucleotides of the invention can be selected to obtain antibodies specifically immunoreactive with that protein and not with other proteins, except for polymorphic variants. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays, Western blots, or immunohistochemistry are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. *See*, Harlow and Lane *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, NY (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Typically, a specific or selective reaction will be at least twice the background signal or noise and more typically more than 10 to 100 times background.

[0099] 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.

[0100] "Anxiety" refers to a physiological and psychological state characterized by any of the following symptoms: heart palpitations, nausea, chest pain, shortness of breath, stomach aches, headaches, panic attacks, sweating, increase in blood pressure, increase in heart rate, increase in the flow of blood to major muscle groups, inhibition of immune and digestive system functions, pale skin, trembling, pupillary dilation, experiencing a sense of dread or panic. An "anxiogenic" substance is one that causes (*e.g.,* brings about, produces, creates, induces) anxiety or increases the level of anxiety in a subject. Anxiogenic effects can be measured by, *e.g.,* the hole-board test in rats and mice (*see, e.g.,* Takeda *et al.*, *Eur. J. Pharm.*, 350:21-29 (1998)). An "anxiolytic" substance is one that may be used for the treatment of symptoms of anxiety in a subject.

[0101] "Risk-taking behavior" refers to the voluntary participation in behaviors that contain, or are at least seen to contain, a significant degree of risk. Generally, a risk is considered to be significant if the behavior involves a high degree of risk in comparison with other equivalent behaviors and involves a high degree of actual risk as measured by the probability of death, injury, financial loss, and the like. Examples of risk-taking behavior include, but are not limited to, health risks such as drinking alcohol, taking drugs, driving fast, or engaging in other addictive behaviors; financial risks such as gambling; participation in high risk sports such as mountain climbing or parachuting; and combinations thereof.

[0102] The term "administering" includes oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release device, *e.g.,* a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.,* buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.,* intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* One skilled in the art will know of additional methods for administering an effective amount of a therapeutic of the present invention.

[0103] The term "mammalian subject" refers to any mammalian species such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock, and the like.

DETAILED DESCRIPTION OF THE INVENTION

[0104] Evidence based on analysis of only a restricted number of molecules suggests altered and unique gene dysregulation 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

[0105] 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.

[0106] 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, preferred embodiments of the invention disclosed herein relate to the mRNA expression levels of genes related to the FGF and GPCRs pathways. Other preferred embodiments focus on the detection of splice variants of FGFR2, which are also dysregulated. In still other embodiments, the invention provides genes which are dysregulated by lithium for the specific treatment of BP disorders.

[0107] The invention also provides nucleic acid sequences and protein sequences which are useful for deciphering the mode of action of currently used mood stabilizers such as lithium.

The sequences provided are also useful for drug discovery, *e.g.*, discovering new leads to identifying more efficacious therapeutic targets in the form of a central molecule/pathway through which an entire system or network of pathways can be modulated to remedy the perturbed cellular process underlying MDD, BP, or a principal endophenotype of these disorders. For instance, manipulating GSK3B can affect one or more of the inositol triphosphate, NF-kB family, mitochondrial apoptosis, and ubiquitin-proteasome pathways. Improved knowledge of target-specificity of drugs could help to minimize side effects associated with numerous mood stabilizers currently in use. The invention provides combinations of biomarkers and relevant genes which can be used in methods for diagnosing MDD, BP and related disorders, as well as for developing additional tools for that purpose, and for monitoring drug efficacy.

[0108] The present invention provides 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. This invention thus provides methods for diagnosis of mental disorders such as mood disorders (*e.g.*, bipolar disorder, major depression, and the like) and other mental disorders having a genetic component by detecting the level of a transcript or translation product of the genes listed in FIGURE 1, FIGURES 5-8, or Tables 1-4, as well as their corresponding biochemical pathways.

[0109] The invention further provides 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. The invention also provides for methods of treating patients with such mental disorders, *e.g.*, by administering the compounds of the invention (*e.g.*, FGF9 protein) or by gene therapy.

[0110] The invention also provides much-needed tools for researching mental illness and the underlying molecular causes of mental illness. These tools include animal models which have been engineered to exhibit phenotypes which are useful for elucidating the molecular basis for mental abnormalities and for identifying treatments for mental abnormalities. For example, the invention provides in one embodiment a mouse with improved memory and learning ability.

[0111] The genes and the polypeptides that they encode, which are associated with mood disorders such as bipolar disease and major depression, are useful for facilitating the design and development of various molecular diagnostic tools such as GeneChips™ containing probe sets specific for all or selected mental disorders, including but not limited to mood disorders, and as an ante-and/or post-natal diagnostic tool for screening newborns in concert with genetic counseling. Other diagnostic applications include evaluation of disease susceptibility, prognosis, and monitoring of disease or treatment process, as well as providing individualized medicine via predictive drug profiling systems, *e.g.*, by correlating specific genomic motifs with the clinical response of a patient to individual drugs. In addition, the present invention is useful for multiplex SNP and haplotype profiling, including but not limited to the identification of therapeutic, diagnostic, and pharmacogenetic targets at the gene, mRNA, protein, and pathway level. Profiling of splice variants and deletions is also useful for diagnostic and therapeutic applications.

[0112] The genes and the polypeptides that they encode, described herein, are also useful as drug targets for the development of therapeutic drugs for the treatment or prevention of mental disorders, including but not limited to mood disorders.

[0113] 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 of the invention. Lithium is drug of choice for treating BP.

[0114] The genes and the polypeptides that they encode, described herein, as also useful as drug targets for the development of therapeutic drugs for the treatment or prevention of mental disorders, including but not limited to mood disorders. Mental disorders have a high co-morbidity with other neurological disorders, such as Parkinson's disease or Alzheimer's. Therefore, the present invention can be used for diagnosis and treatment of patients with multiple disease states that include a mental disorder such as a mood disorder. These mood disorders include BP, MDD, and other disorders such as psychotic-depression, depression and anxiety features, melancholic depression, chronic depression, BPI and BPII.

II. General Recombinant Nucleic Acid Methods for Use with the Invention

[0115] In numerous embodiments of the present invention, polynucleotides of the invention will 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 of the invention in different species, for diagnostic purposes in a patient, *e.g.*, to detect mutations or to detect expression levels of nucleic acids or polypeptides of the invention. In some embodiments, the sequences of the invention are operably linked to a heterologous promoter. In one embodiment, the nucleic acids of the invention are from any mammal, including, in particular, *e.g.*, a human, a mouse, a rat, a primate, *etc.*

A. General Recombinant Nucleic Acids Methods

[0116] This invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (3rd ed. 2001); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994)).

[0117] 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.

[0118] 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).

[0119] 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

[0120] 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.

[0121] 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 of the invention.

[0122] 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).

[0123] 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 of the invention. 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 of the invention 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.

[0124] Appropriate primers and probes for identifying polynucleotides of the invention 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).

[0125] 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.

[0126] A gene encoding a polypeptide of the invention 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 of the Invention

[0127] Either naturally occurring or recombinant polypeptides of the invention can be purified for use in functional assays. Naturally occurring polypeptides, *e.g.*, FGF9, and/or 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.

[0128] The polypeptides of the invention 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*).

[0129] 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 of the invention. 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

[0130] 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.

[0131] 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), l 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.

[0132] 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.

[0133] 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. Solubility Fractionation

[0134] 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 of proteins, 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. Size Differential Filtration

[0135] 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. Column Chromatography

[0136] 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.

[0137] 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. RNA Interference Methods for Use with the Invention

[0138] RNA interference (RNAi) is an evolutionarily conserved process in which recognition of double stranded RNA (dsRNA) ultimately leads to post-transcriptional suppression of gene expression. This suppression is mediated by short dsRNA, also called small-interfering RNA (siRNA), which induces specific degradation of mRNA through complementary base pairing. In several model systems, this natural response has been developed into a powerful tool for the investigation of gene function (*see, e.g.,* Elbashir *et al.*, *Genes Dev.*, 15:188-200 (2001); Hammond *et al.*, *Nat. Rev. Genet.*, 2:110-119 (2001)). More recently, it was discovered that introducing synthetic 21-nucleotide dsRNA duplexes into

mammalian cells could efficiently silence gene expression. Although the precise mechanism is still unclear, RNAi offers a new way to inactivate genes of interest.

[0139] In certain aspects, the siRNA molecules of the invention comprise a sequence of about 15-60, 15-50, 15-40, 15-30, 15-25, or 19-25 (duplex) nucleotides in length, and more typically about 20-24, 21-22, or 21-23 (duplex) nucleotides in length. siRNA duplexes may comprise 3' overhangs of about 1 to about 4 nucleotides or about 2 to about 3 nucleotides and 5' phosphate termini. The siRNA molecule may have substantial or complete identity to the target gene or may comprise a region of mismatch (*i.e.*, a mismatch motif). Examples of siRNA include, without limitation, a double-stranded polynucleotide molecule assembled from two separate stranded molecules, wherein one strand is the sense strand and the other is the complementary antisense strand; a double-stranded polynucleotide molecule assembled from a single stranded molecule, where the sense and antisense regions are linked by a nucleic acid-based or non-nucleic acid-based linker; a double-stranded polynucleotide molecule with a hairpin secondary structure having self-complementary sense and antisense regions; and a circular single-stranded polynucleotide molecule with two or more loop structures and a stem having self-complementary sense and antisense regions, where the circular polynucleotide can be processed *in vivo* or *in vitro* to generate an active double stranded siRNA molecule.

[0140] Suitable siRNA sequences can be identified using any means known in the art. Typically, the methods described in Elbashir *et al.*, *Nature,* 411:494-498 (2001) and Elbashir *et al.*, *EMBO J.,* 20:6877-6888 (2001) are combined with rational design rules set forth in Reynolds *et al.*, *Nature Biotech.,* 22(3):326-330 (2004).

[0141] siRNA can be provided in several forms including, *e.g.*, as one or more isolated duplexes, as longer dsRNA, or as siRNA or dsRNA transcribed from a transcriptional cassette in a DNA plasmid. The siRNA sequences may have overhangs (*e.g.*, 3' or 5' overhangs as described in Elbashir *et al.*, *Genes Dev.*, 15:188 (2001) or Nykänen *et al.*, *Cell*, 107:309 (2001), or may lack overhangs (*i.e.*, to have blunt ends).

[0142] An RNA population can be used to provide long precursor RNAs, or long precursor RNAs that have substantial or complete identity to a selected target sequence can be used to make the siRNA. The RNAs can be isolated from cells or tissue, synthesized, and/or cloned according to methods well known to those of skill in the art. The RNA can be a mixed

population (obtained from cells or tissue, transcribed from cDNA, subtracted, selected, *etc.*), or can represent a single target sequence. RNA can be naturally occurring (*e.g.*, isolated from tissue or cell samples), synthesized *in vitro (e.g.,* using T7 or SP6 polymerase and PCR products or a cloned cDNA), or chemically synthesized.

[0143] To form a long dsRNA, for synthetic RNAs, the complement is also transcribed *in vitro* and hybridized to form a dsRNA. If a naturally-occurring RNA population is used, the RNA complements are also provided (*e.g.*, to form dsRNA for digestion by *E. coli* RNAse III or Dicer), *e.g.*, by transcribing cDNAs corresponding to the RNA population, or by using RNA polymerases. The precursor RNAs are then hybridized to form double stranded RNAs for digestion. The dsRNAs can be directly administered to a subject or can be digested *in vitro* prior to administration.

[0144] Methods for isolating RNA, synthesizing RNA, hybridizing nucleic acids, making and screening cDNA libraries, and performing PCR are well known in the art (*see, e.g.,* Gubler and Hoffman, *Gene*, 25:263-269 (1983); Sambrook *et al., supra*; Ausubel *et al., supra*), as are PCR methods (*see,* U.S. Patents 4,683,195 and 4,683,202; Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications* (1990)). Expression libraries are also well known to those of skill in the art. Additional basic texts disclosing the general methods of use in this invention include Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994).

[0145] In some embodiments, the siRNA molecules are chemically synthesized. The oligonucleotides that comprise the siRNA can be synthesized using any of a variety of techniques known in the art, such as those described in Usman *et al.*, *J. Am. Chem. Soc.*, 109:7845 (1987); Scaringe *et al.*, *Nucl. Acids Res.*, 18:5433 (1990); Wincott *et al.*, *Nucl. Acids Res.*, 23:2677-2684 (1995); and Wincott *et al., Methods Mol. Bio.*, 74:59 (1997). The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end and phosphoramidites at the 3'-end. As a non-limiting example, small scale syntheses can be conducted on an Applied Biosystems synthesizer using a 0.2 mol scale protocol. Alternatively, syntheses at the 0.2 mol scale can be performed on a 96-well plate synthesizer from Protogene (Palo Alto, CA). However, a larger or smaller scale of synthesis is also within the scope of the present invention. Suitable

reagents for oligonucleotide synthesis, methods for RNA deprotection, and methods for RNA purification are known to those of skill in the art.

[0146] The siRNA molecules of the invention can also be synthesized via a tandem synthesis technique, wherein both strands are synthesized as a single continuous oligonucleotide fragment or strand separated by a cleavable linker that is subsequently cleaved to provide separate fragments or strands that hybridize to form the siRNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siRNA can be readily adapted to both multiwell/multiplate synthesis platforms as well as large scale synthesis platforms employing batch reactors, synthesis columns, and the like. Alternatively, siRNA molecules can be assembled from two distinct oligonucleotides, wherein one oligonucleotide comprises the sense strand and the other comprises the antisense strand of the siRNA. For example, each strand can be synthesized separately and joined together by hybridization or ligation following synthesis and/or deprotection. In certain other instances, siRNA molecules can be synthesized as a single continuous oligonucleotide fragment, where the self-complementary sense and antisense regions hybridize to form an siRNA duplex having hairpin secondary structure.

[0147] In certain instances, the siRNA contains nucleotide analogs or modified backbone residues or linkages, which may be synthetic, naturally-occurring, and/or non-naturally occurring, and the modified siRNA exhibits similar binding properties as the unmodified (reference) siRNA. Examples of phosphate backbone modifications include, but are not limited to, phosphorothioates, phosphoramidates, methyl phosphonates, and chiral-methyl phosphonates (*see, e.g.,* Hunziker *et al.*, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331-417 (1995); Mesmaeker *et al.*, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39 (1994)). Non-limiting examples of modified nucleotides include ribonucleotides having a 2'-O-methyl (2'OMe), 2'-deoxy-2'-fluoro (2'F), 2'-deoxy, 5-C-methyl, 2'-O-(2-methoxyethyl) (MOE), 4'-thio, 2'-amino, or 2'-C-allyl group. Additional examples of modified nucleotides and types of chemical modifications that can be introduced into siRNA are described, *e.g.,* in Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag Ed. (1984), Loakes, *Nucl. Acids Res.,* 29:2437-2447 (2001), UK Patent No. GB 2,397,818 B, and U.S. Patent Publication Nos. 20040192626, 20050282188, and 20070135372. In some

embodiments, the siRNA may further comprise terminal cap moieties as described in U.S. Patent No. 5,998,203 and Beaucage *et al.*, *Tetrahedron* 49:1925 (1993).

[0148] The siRNA can optionally comprise one or more non-nucleotides in one or both strands. As used herein, the term "non-nucleotide" refers to any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base such as adenosine, guanine, cytosine, uracil, or thymine and therefore lacks a base at the 1'-position.

[0149] In other embodiments, the siRNA may comprise a conjugate attached thereto. The conjugate can be attached at the 5' and/or 3'-end of the sense and/or antisense strand of the siRNA via a covalent attachment such as, *e.g.,* a biodegradable linker. The conjugate can also be attached to the siRNA, *e.g.,* through a carbamate group or other linking group (*see, e.g.,* U.S. Patent Publication Nos. 20050074771, 20050043219, and 20050158727). In certain instances, the conjugate is a molecule that facilitates the delivery of the siRNA into a cell. In certain other instances, the conjugate is a molecule that increases the stability of the siRNA upon administration to a mammalian subject. Examples of conjugate molecules suitable for attachment to siRNA include, without limitation, steroids such as cholesterol, glycols such as polyethylene glycol (PEG), human serum albumin (HSA), fatty acids, carotenoids, terpenes, bile acids, folates, sugars (*e.g.,* galactose, galactosamine, N-acetyl galactosamine, glucose, mannose, fructose, fucose, *etc.*), phospholipids, peptides, ligands for cellular receptors capable of mediating cellular uptake, and combinations thereof (*see, e.g.,* U.S. Patent Publication Nos. 20030130186, 20040110296, and 20040249178; U.S. Patent No. 6,753,423). The type of conjugate used and the extent of conjugation to the siRNA molecule can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of the siRNA while retaining RNAi activity. As such, one skilled in the art can screen siRNA having various conjugates attached thereto to identify ones having improved properties and full RNAi activity using any of a variety of well-known *in vitro* cell culture or *in vivo* animal models.

[0150] The siRNA molecules of the invention may be administered using any carrier system known to one skilled in the art. Examples of carrier systems suitable for delivering the siRNA to a target cell, tissue, or organ of interest include, but are not limited to, lipid-

based carrier systems such as stabilized nucleic acid-lipid particles, cationic lipid- or liposome-nucleic acid complexes (*i.e.,* lipoplexes), liposomes, micelles, virosomes, or mixtures thereof; polymer-based carrier systems such as cationic polymer-nucleic acid complexes (*i.e.,* polyplexes); cyclodextrin-based carrier systems such as a cyclodextrin polymer-nucleic acid complexes; and protein-based carrier systems such as cationic peptide-nucleic acid complexes. One skilled in the art will appreciate that the siRNA molecule of the invention can also be delivered as naked siRNA, *e.g.,* by direct injection into a subject.

[0151] Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.,* U.S. Patent Nos. 5,753,613; 5,785,992; 5,705,385; 5,976,567; 5,981,501; 6,110,745; and 6,320,017; and PCT Publication No. WO 96/40964. Additional lipid-based carrier systems are described in U.S. Patent Publication No. 20030203865 and Zhang *et al.*, *J. Control Release*, 100:165-180 (2004) (lipoplexes), U.S. Patent Publication No. 20020192275 (pH-sensitive lipoplexes), U.S. Patent Publication Nos. 20030180950 (reversibly masked lipoplexes), U.S. Patent No. 6,756,054 and U.S. Patent Publication No. 20050234232 (cationic lipid-based compositions), U.S. Patent Publication Nos. 20030229040, 20020160038, and 20020012998, U.S. Patent No. 5,908,635, and PCT Publication No. WO 01/72283 (cationic liposomes), U.S. Patent Publication No. 20030026831 (anionic liposomes), U.S. Patent Publication No. 20020192274 and AU 2003210303 (pH-sensitive liposomes), U.S. Patent Publication No. 20030108597 and PCT Publication No. WO 00/50008 (antibody-coated liposomes), U.S. Patent Publication No. 20030198664 (cell-type specific liposomes), U.S. Patent No. 6,207,456 (liposomes containing nucleic acid and peptides), U.S. Patent Publication No. 20030031704 (liposomes containing lipids derivatized with releasable hydrophilic polymers), PCT Publication Nos. WO 03/057190 and WO 03/059322 (lipid-entrapped nucleic acid), U.S. Patent Publication No. 20030129221 and U.S. Patent No. 5,756,122 (lipid-encapsulated nucleic acid), U.S. Patent Publication Nos. 20030035829 and 20030072794, and U.S. Patent No. 6,200,599 (other liposomal compositions), EP1304160 (stabilized mixtures of liposomes and emulsions), and U.S. Patent Publication No. 20050037086 (emulsion compositions).

[0152] Examples of polymer-based carrier systems include, but are not limited to, cationic polymer-nucleic acid complexes (*i.e.,* polyplexes). To form a polyplex, a nucleic acid (*e.g.,* siRNA) is typically complexed with a cationic polymer having a linear, branched, star, or dendritic polymeric structure that condenses the nucleic acid into positively charged particles

capable of interacting with anionic proteoglycans at the cell surface and entering cells by endocytosis. In some embodiments, the polyplex comprises the siRNA complexed with a cationic polymer such as polyethylenimine (PEI) (*see, e.g.,* U.S. Patent No. 6,013,240; commercially available from Qbiogene, Inc. (Carlsbad, CA) as *In vivo* jetPEI™, a linear form of PEI), polypropylenimine (PPI), polyvinylpyrrolidone (PVP), poly-L-lysine (PLL), diethylaminoethyl (DEAE)-dextran, poly(β-amino ester) (PAE) polymers (*see, e.g.,* Lynn *et al.*, *J. Am. Chem. Soc.*, 123:8155-8156 (2001)), chitosan, polyamidoamine (PAMAM) dendrimers (*see, e.g.,* Kukowska-Latallo *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:4897-4902 (1996)), porphyrin (*see, e.g.,* U.S. Patent No. 6,620,805), polyvinylether (*see, e.g.,* U.S. Patent Publication No. 20040156909), polycyclic amidinium (*see, e.g.,* U.S. Patent Publication No. 20030220289), other polymers comprising primary amine, imine, guanidine, and/or imidazole groups (*see, e.g.,* U.S. Patent No. 6,013,240; PCT Publication No. WO/9602655; PCT Publication No. WO95/21931; Zhang *et al.*, *J. Control Release*, 100:165-180 (2004); and Tiera *et al.*, *Curr. Gene Ther.*, 6:59-71 (2006)), and a mixture thereof. In other embodiments, the polyplex comprises cationic polymer-nucleic acid complexes as described in U.S. Patent Publication Nos. 20060211643, 20050222064, 20030125281, and 20030185890, and PCT Publication No. WO 03/066069; biodegradable poly(β-amino ester) polymer-nucleic acid complexes as described in U.S. Patent Publication No. 20040071654; microparticles containing polymeric matrices as described in U.S. Patent Publication No. 20040142475; other microparticle compositions as described in U.S. Patent Publication No. 20030157030; condensed nucleic acid complexes as described in U.S. Patent Publication No. 20050123600; and nanocapsule and microcapsule compositions as described in AU 2002358514 and PCT Publication No. WO 02/096551.

[0153] The siRNA may alternatively be complexed with cyclodextrin or a polymer thereof. Non-limiting examples of cyclodextrin-based carrier systems include the cyclodextrin-modified polymer-nucleic acid complexes described in U.S. Patent Publication No. 20040087024; the linear cyclodextrin copolymer-nucleic acid complexes described in U.S. Patent Nos. 6,509,323, 6,884,789, and 7,091,192; and the cyclodextrin polymer-complexing agent-nucleic acid complexes described in U.S. Patent No. 7,018,609. In certain instances, the siRNA may be complexed with a peptide or polypeptide. An example of a protein-based carrier system includes, but is not limited to, the cationic oligopeptide-nucleic acid complex described in PCT Publication No. WO95/21931.

V. Detection of Gene Expression

[0154] Those of skill in the art will recognize that detection of expression of polynucleotides of the invention has many uses. For example, as discussed herein, detection of the level of polypeptides or polynucleotides of the invention in a patient is useful for diagnosing mood disorders or psychotic disorders or a predisposition for a mood disorder or psychotic disorders. Moreover, detection of gene expression is useful to identify modulators of expression of the polypeptides or polynucleotides of the invention.

[0155] A variety of methods of specific DNA and RNA measurement using nucleic acid hybridization techniques are known to those of skill in the art (*see*, Sambrook, *supra*). Some methods involve an electrophoretic separation (*e.g.*, Southern blot for detecting DNA, and Northern blot for detecting RNA), but measurement of DNA and RNA can also be carried out in the absence of electrophoretic separation (*e.g.*, by dot blot). Southern blot of genomic DNA (*e.g.*, from a human) can be used for screening for restriction fragment length polymorphism (RFLP) to detect the presence of a genetic disorder affecting a polypeptide of the invention.

[0156] The selection of a nucleic acid hybridization format is not critical. A variety of nucleic acid hybridization formats are known to those skilled in the art. For example, common formats include sandwich assays and competition or displacement assays. Hybridization techniques are generally described in Hames and Higgins *Nucleic Acid Hybridization, A Practical Approach*, IRL Press (1985); Gall and Pardue, *Proc. Natl. Acad. Sci. U.S.A.*, 63:378-383 (1969); and John *et al. Nature*, 223:582-587 (1969).

[0157] Detection of a hybridization complex may require the binding of a signal-generating complex to a duplex of target and probe polynucleotides or nucleic acids. Typically, such binding occurs through ligand and anti-ligand interactions as between a ligand-conjugated probe and an anti-ligand conjugated with a signal. The binding of the signal generation complex is also readily amenable to accelerations by exposure to ultrasonic energy.

[0158] The label may also allow indirect detection of the hybridization complex. For example, where the label is a hapten or antigen, the sample can be detected by using antibodies. In these systems, a signal is generated by attaching fluorescent or enzyme molecules to the antibodies or in some cases, by attachment to a radioactive label (*see*, *e.g.*, Tijssen, "*Practice and Theory of Enzyme Immunoassays*," *Laboratory Techniques in*

Biochemistry and Molecular Biology, Burdon and van Knippenberg Eds., Elsevier (1985), pp. 9-20).

[0159] The probes are typically labeled either directly, as with isotopes, chromophores, lumiphores, chromogens, or indirectly, such as with biotin, to which a streptavidin complex may later bind. Thus, the detectable labels used in the assays of the present invention can be primary labels (where the label comprises an element that is detected directly or that produces a directly detectable element) or secondary labels (where the detected label binds to a primary label, *e.g.*, as is common in immunological labeling). Typically, labeled signal nucleic acids are used to detect hybridization. Complementary nucleic acids or signal nucleic acids may be labeled by any one of several methods typically used to detect the presence of hybridized polynucleotides. The most common method of detection is the use of autoradiography with ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P-labeled probes or the like.

[0160] Other labels include, *e.g.*, ligands that bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden *Introduction to Immunocytochemistry*, 2nd ed., Springer Verlag, NY (1997); and in Haugland *Handbook of Fluorescent Probes and Research Chemicals*, a combined handbook and catalogue Published by Molecular Probes, Inc. (1996).

[0161] In general, a detector which monitors a particular probe or probe combination is used to detect the detection reagent label. Typical detectors include spectrophotometers, phototubes and photodiodes, microscopes, scintillation counters, cameras, film and the like, as well as combinations thereof. Examples of suitable detectors are widely available from a variety of commercial sources known to persons of skill in the art. Commonly, an optical image of a substrate comprising bound labeling moieties is digitized for subsequent computer analysis.

[0162] Most typically, the amount of RNA is measured by quantifying the amount of label fixed to the solid support by binding of the detection reagent. Typically, the presence of a modulator during incubation will increase or decrease the amount of label fixed to the solid support relative to a control incubation which does not comprise the modulator, or as

compared to a baseline established for a particular reaction type. Means of detecting and quantifying labels are well known to those of skill in the art.

[0163] In preferred embodiments, the target nucleic acid or the probe is immobilized on a solid support. Solid supports suitable for use in the assays of the invention are known to those of skill in the art. As used herein, a solid support is a matrix of material in a substantially fixed arrangement.

[0164] A variety of automated solid-phase assay techniques are also appropriate. For instance, very large scale immobilized polymer arrays (VLSIPS™), available from Affymetrix, Inc. (Santa Clara, CA) can be used to detect changes in expression levels of a plurality of genes involved in the same regulatory pathways simultaneously. *See*, Tijssen, *supra.*, Fodor *et al.* (1991) *Science*, 251: 767- 777; Sheldon *et al.* (1993) *Clinical Chemistry* 39(4): 718-719, and Kozal *et al.* (1996) *Nature Medicine* 2(7): 753-759.

[0165] Detection can be accomplished, for example, by using a labeled detection moiety that binds specifically to duplex nucleic acids (*e.g.*, an antibody that is specific for RNA-DNA duplexes). One preferred example uses an antibody that recognizes DNA-RNA heteroduplexes in which the antibody is linked to an enzyme (typically by recombinant or covalent chemical bonding). The antibody is detected when the enzyme reacts with its substrate, producing a detectable product. Coutlee *et al.* (1989) *Analytical Biochemistry* 181:153-162; Bogulavski (1986) *et al. J. Immunol. Methods* 89:123-130; Prooijen-Knegt (1982) *Exp. Cell Res.* 141:397-407; Rudkin (1976) *Nature* 265:472-473, Stollar (1970) *Proc. Nat'l Acad. Sci. USA* 65:993-1000; Ballard (1982) *Mol. Immunol.* 19:793-799; Pisetsky and Caster (1982) *Mol. Immunol.* 19:645-650; Viscidi *et al.* (1988) *J. Clin. Microbial.* 41:199-209; and Kiney *et al.* (1989) *J. Clin. Microbiol.* 27:6-12 describe antibodies to RNA duplexes, including homo and heteroduplexes. Kits comprising antibodies specific for DNA:RNA hybrids are available, *e.g.*, from Digene Diagnostics, Inc. (Beltsville, MD).

[0166] In addition to available antibodies, one of skill in the art can easily make antibodies specific for nucleic acid duplexes using existing techniques, or modify those antibodies that are commercially or publicly available. In addition to the art referenced above, general methods for producing polyclonal and monoclonal antibodies are known to those of skill in the art (*see, e.g.,* Paul (3rd ed.) *Fundamental Immunology* Raven Press, Ltd., NY (1993); Coligan *Current Protocols in Immunology* Wiley/Greene, NY (1991); Harlow and Lane

Antibodies: A Laboratory Manual Cold Spring Harbor Press, NY (1988); Stites *et al.* (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Goding *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, NY, (1986); and Kohler and Milstein *Nature* 256: 495-497 (1975)). Other suitable techniques for antibody preparation include selection of libraries of recombinant antibodies in phage or similar vectors (*see*, Huse *et al. Science* 246:1275-1281 (1989); and Ward *et al. Nature* 341:544-546 (1989)). Specific monoclonal and polyclonal antibodies and antisera will usually bind with a K_D of at least about 0.1 μM, preferably at least about 0.01 μM or better, and most typically and preferably, 0.001 μM or better.

[0167] The nucleic acids used in this invention can be either positive or negative probes. Positive probes bind to their targets and the presence of duplex formation is evidence of the presence of the target. Negative probes fail to bind to the suspect target and the absence of duplex formation is evidence of the presence of the target. For example, the use of a wild type specific nucleic acid probe or PCR primers may serve as a negative probe in an assay sample where only the nucleotide sequence of interest is present.

[0168] The sensitivity of the hybridization assays may be enhanced through use of a nucleic acid amplification system that multiplies the target nucleic acid being detected. Examples of such systems include the polymerase chain reaction (PCR) system, in particular RT-PCR or real time PCR, and the ligase chain reaction (LCR) system. Other methods recently described in the art are the nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario) and Q Beta Replicase systems. These systems can be used to directly identify mutants where the PCR or LCR primers are designed to be extended or ligated only when a selected sequence is present. Alternatively, the selected sequences can be generally amplified using, for example, nonspecific PCR primers and the amplified target region later probed for a specific sequence indicative of a mutation.

[0169] An alternative means for determining the level of expression of the nucleic acids of the present invention is *in situ* hybridization. *In situ* hybridization assays are well known and are generally described in Angerer *et al., Methods Enzymol.* 152:649-660 (1987). In an *in situ* hybridization assay, cells, preferentially human cells from the cerebellum or the hippocampus, are fixed to a solid support, typically a glass slide. If DNA is to be probed, the cells are denatured with heat or alkali. The cells are then contacted with a hybridization

solution at a moderate temperature to permit annealing of specific probes that are labeled. The probes are preferably labeled with radioisotopes or fluorescent reporters.

VI. Immunological Detection of the Polypeptides of the Invention

[0170] In addition to the detection of polynucleotide expression using nucleic acid hybridization technology, one can also use immunoassays to detect polypeptides of the invention. Immunoassays can be used to qualitatively or quantitatively analyze polypeptides. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

A. Antibodies to Target Polypeptides or Other Immunogens

[0171] Methods for producing polyclonal and monoclonal antibodies that react specifically with a protein of interest or other immunogen are known to those of skill in the art (*see, e.g.,* Coligan, *supra*; and Harlow and Lane, *supra*; Stites *et al.*, *supra* and references cited therein; Goding, *supra*; and Kohler and Milstein *Nature*, 256:495-497 (1975)). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors (*see*, Huse *et al.*, *supra*; and Ward *et al.*, *supra*). For example, in order to produce antisera for use in an immunoassay, the protein of interest or an antigenic fragment thereof, is isolated as described herein. For example, a recombinant protein is produced in a transformed cell line. An inbred strain of mice or rabbits is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen.

[0172] Polyclonal sera are collected and titered against the immunogen in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross-reactivity against unrelated proteins or even other homologous proteins from other organisms, using a competitive binding immunoassay. Specific monoclonal and polyclonal antibodies and antisera will usually bind with a K_D of at least about 0.1 mM, more usually at least about 1 μM, preferably at least about 0.1 μM or better, and most preferably, 0.01 μM or better.

[0173] A number of proteins of the invention comprising immunogens may be used to produce antibodies specifically or selectively reactive with the proteins of interest. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein, such as one comprising an amino acid sequence encoded by a gene listed in Tables 1-4 and FIGURE 1 may also be used either in pure or impure form. Synthetic peptides made using the protein sequences described herein may also be used as an immunogen for the production of antibodies to the protein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells and purified as generally described *supra*. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated for subsequent use in immunoassays to measure the protein.

[0174] Methods of production of polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the polypeptide of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (*see*, Harlow and Lane, *supra*).

[0175] Monoclonal antibodies may be obtained using various techniques familiar to those of skill in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (s*ee*, Kohler and Milstein, *Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include, *e.g.*, transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse *et al.*, *supra*.

[0176] Once target protein specific antibodies are available, the protein can be measured by a variety of immunoassay methods with qualitative and quantitative results available to the clinician. For a review of immunological and immunoassay procedures in general *see*, Stites, *supra*. Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in Maggio *Enzyme Immunoassay*, CRC Press, Boca Raton, Florida (1980); Tijssen, *supra*; and Harlow and Lane, *supra*.

[0177] Immunoassays to measure target proteins in a human sample may use a polyclonal antiserum that was raised to the protein (*e.g.*, one has an amino acid sequence encoded by a gene listed FIGURE 1, FIGURES 5-8, or Tables 1-4) or a fragment thereof. This antiserum is selected to have low cross-reactivity against different proteins and any such cross-reactivity is removed by immunoabsorption prior to use in the immunoassay.

B. Immunological Binding Assays

[0178] In a preferred embodiment, a protein of interest is detected and/or quantified using any of a number of well-known immunological binding assays (*see, e.g.,* U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, *see* also Asai *Methods in Cell Biology Volume 37: Antibodies in Cell Biology*, Academic Press, Inc. NY (1993); Stites, *supra*. Immunological binding assays (or immunoassays) typically utilize a "capture agent" to specifically bind to and often immobilize the analyte (in this case a polypeptide of the present invention or antigenic subsequences thereof). The capture agent is a moiety that specifically binds to the analyte. In a preferred embodiment, the capture agent is an antibody that specifically binds, for example, a polypeptide of the invention. The antibody may be produced by any of a number of means well known to those of skill in the art and as described above.

[0179] Immunoassays also often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/protein complex.

[0180] In a preferred embodiment, the labeling agent is a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is

derived. The second antibody can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

[0181] Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G, can also be used as the label agents. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (*see,* generally, Kronval, *et al. J. Immunol.*, 111:1401-1406 (1973); and Akerstrom, *et al. J. Immunol.*, 135:2589-2542 (1985)).

[0182] Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. The incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

1. Non-Competitive Assay Formats

[0183] Immunoassays for detecting proteins of interest from tissue samples may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (in this case the protein) is directly measured. In one preferred "sandwich" assay, for example, the capture agent (*e.g.*, antibodies specific for a polypeptide encoded by a gene listed in FIGURE 1, FIGURES 5-8, or Tables 1-4) can be bound directly to a solid substrate where it is immobilized. These immobilized antibodies then capture the polypeptide present in the test sample. The polypeptide thus immobilized is then bound by a labeling agent, such as a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

2. Competitive Assay Formats

[0184] In competitive assays, the amount of analyte (such as a polypeptide encoded by a gene listed in FIGURE 1, FIGURES 5-8, or Tables 1-4) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte displaced (or competed

away) from a capture agent (*e.g.*, an antibody specific for the analyte) by the analyte present in the sample. In one competitive assay, a known amount of, in this case, the protein of interest is added to the sample and the sample is then contacted with a capture agent, in this case an antibody that specifically binds to a polypeptide of the invention. The amount of immunogen bound to the antibody is inversely proportional to the concentration of immunogen present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. For example, the amount of the polypeptide bound to the antibody may be determined either by measuring the amount of subject protein present in a protein/antibody complex or, alternatively, by measuring the amount of remaining uncomplexed protein. The amount of protein may be detected by providing a labeled protein molecule.

[0185] Immunoassays in the competitive binding format can be used for cross-reactivity determinations. For example, a protein of interest can be immobilized on a solid support. Proteins are added to the assay which compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to that of the protein of interest. The percent cross-reactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% cross-reactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are optionally removed from the pooled antisera by immunoabsorption with the considered proteins, *e.g.*, distantly related homologs.

[0186] The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein, thought to be perhaps a protein of the present invention, to the immunogen protein. In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than 10 times the amount of the protein partially encoded by a sequence herein that is required, then the second protein is said to specifically bind to an antibody generated to an immunogen consisting of the target protein.

3. Other Assay Formats

[0187] In a particularly preferred embodiment, western blot (immunoblot) analysis is used to detect and quantify the presence of a polypeptide of the invention in the sample. The

technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support (such as, *e.g.*, a nitrocellulose filter, a nylon filter, or a derivatized nylon filter) and incubating the sample with the antibodies that specifically bind the protein of interest. For example, the antibodies specifically bind to a polypeptide of interest on the solid support. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (*e.g.*, labeled sheep anti-mouse antibodies) that specifically bind to the antibodies against the protein of interest.

[0188] Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (*e.g.*, antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (*see*, Monroe *et al*. (1986) *Amer. Clin. Prod. Rev.* 5:34-41).

4. Labels

[0189] The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well developed in the field of immunoassays and, in general, most labels useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (*e.g.*, Dynabeads^{TM}), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, *etc.*) beads.

[0190] The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, the ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[0191] Non-radioactive labels are often attached by indirect means. The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorescent compound. A variety of enzymes and fluorescent compounds can be used with the methods of the present invention and are well-known to those of skill in the art (for a review of various labeling or signal producing systems which may be used, *see*, *e.g.*, U.S. Patent No. 4,391,904).

[0192] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge-coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected directly by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[0193] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need to be labeled and the presence of the target antibody is detected by simple visual inspection.

[0194] In some embodiments, BP or MDD in a patient may be diagnosed or otherwise evaluated by visualizing expression *in situ* of one or more of the appropriately dysregulated gene sequences identified herein, *e.g.*, FIGURE 1, FIGURES 5-8, or Tables 1-4. Those skilled in the art of visualizing the presence or expression of molecules including nucleic acids, polypeptides and other biochemicals in the brains of living patients will appreciate that the gene expression information described herein may be utilized in the context of a variety of visualization methods. Such methods include, but are not limited to, single-photon emission-computed tomography (SPECT) and positron-emitting tomography (PET) methods. *See*, *e.g.*, Vassaux and Groot-wassink, "In Vivo Noninvasive Imaging for Gene Therapy," J. Biomedicine and Biotechnology, 2: 92-101 (2003).

[0195] PET and SPECT imaging shows the chemical functioning of organs and tissues, while other imaging techniques – such as X-ray, CT and MRI – show structure. The use of PET and SPECT imaging is useful for qualifying and monitoring the development of brain diseases, including bipolar disorder, major depression disorder, schizophrenia and associated disorders. In some instances, the use of PET or SPECT imaging allows diseases to be detected years earlier than the onset of symptoms. The use of small molecules for labelling and visualizing the presence or expression of polypeptides and nucleotides has had success, for example, in visualizing proteins in the brains of Alzheimer's patients, as described by, *e.g.*, Herholz K *et al.*, Mol Imaging Biol., 6(4):239-69 (2004); Nordberg A, Lancet Neurol., 3(9):519-27 (2004); Neuropsychol Rev., Zakzanis KK *et al.*, 13(1):1-18 (2003); Kung MP *et al*, Brain Res.,1025(1-2):98-105 (2004); and Herholz K, Ann Nucl Med., 17(2):79-89 (2003).

[0196] The dysregulated genes disclosed in FIGURE 1, FIGURES 5-8, or Tables 1-4, or their encoded peptides (if any), or fragments thereof, can be used in the context of PET and SPECT imaging applications. After modification with appropriate tracer residues for PET or SPECT applications, molecules which interact or bind with the transcripts in Tables FIGURE 1, FIGURES 5-8, or Tables 1-4 or with any polypeptides encoded by those transcripts may be used to visualize the patterns of gene expression and facilitate diagnosis of schizophrenia, MDD, BP, and related disorders as described herein. Similarly, if the encoded polypeptides encode enzymes, labeled molecules which interact with the products of catalysis by the enzyme may be used for the *in vivo* imaging and diagnostic application described herein.

[0197] Antisense technology is particularly suitable for detecting the the transcripts identified in FIGURE 1, FIGURES 5-8, or Tables 1-4. For example, the use of antisense peptide nucleic acid (PNA) labeled with an appropriate radionuclide, such as ^{111}In, and conjugated to a brain drug-targeting system to enable transport across biologic membrane barriers, has been demonstrated to allow imaging of endogenous gene expression in brain cancer. *See* Suzuki *et al.*, Journal of Nuclear Medicine, 10:1766-1775 (2004). Suzuki *et al.* utilize a delivery system comprising monoclonal antibodies that target transferring receptors at the blood-brain barrier and facilitate transport of the PNA across that barrier. Modified embodiments of this technique may be used to target upregulated genes associated with schizophrenia, BP or MDD, such as the upregulated genes which appear in FIGURE 1, FIGURES 5-8, or Tables 1-4, in methods of treating schizophrenic, BP or MDD patients.

[0198] In other embodiments, the dysregulated genes listed in FIGURE 1, FIGURES 5-8, or Tables 1-4 may be used in the context of prenatal and neonatal diagnostic methods. For example, fetal or neonatal samples can be obtained and the expression levels of appropriate transcripts (*e.g.*, the transcripts in FIGURE 1, FIGURES 5-8, or Tables 1-4) may be measured and correlated with the presence or increased likelihood of a mental disorder, *e.g.*, MDD. Similarly, the presence of one or more of the SNPs identified in FIGURE 1 may be used to infer or corroborate dysregulated expression of a gene and the likelihood of a mood disorder in prenatal, neonatal, children and adult patients.

[0199] In other embodiments, the brain labeling and imaging techniques described herein or variants thereof may be used in conjunction with any of the dysregulated gene sequences in FIGURE 1, FIGURES 5-8, or Tables 1-4 in a forensic analysis, *i.e.*, to determine whether a deceased individual suffered from schizophrenia, BP, or MDD.

VII. Screening for Modulators of Polypeptides and Polynucleotides of the Invention

[0200] Modulators of polypeptides or polynucleotides of the invention, *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 of the invention can be used to treat patients with mood disorders or psychotic disorders.

A. Screening Methods

[0201] A number of different screening protocols can be utilized to identify agents that modulate the level of expression or activity of polypeptides and polynucleotides of the invention 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 of the invention, modulating inhibitor binding to the polypeptide or activating expression of the polypeptide or polynucleotide, for example.

1. Binding Assays

[0202] Preliminary screens can be conducted by screening for agents capable of binding to a polypeptide of the invention, as at least some of the agents so identified are likely

modulators of polypeptide activity. The binding assays usually involve contacting a polypeptide of the invention 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.

[0203] Binding assays are also useful, *e.g.*, for identifying endogenous proteins that interact with a polypeptide of the invention. For example, antibodies, receptors or other molecules that bind a polypeptide of the invention can be identified in binding assays.

2. Expression Assays

[0204] Certain screening methods involve screening for a compound that up or down-regulates the expression of a polypeptide or polynucleotide of the invention. Such methods generally involve conducting cell-based assays in which test compounds are contacted with one or more cells expressing a polypeptide or polynucleotide of the invention and then detecting an increase or decrease in expression (either transcript, translation product, or catalytic product). Some assays are performed with peripheral cells, or other cells, that express an endogenous polypeptide or polynucleotide of the invention.

[0205] Polypeptide or polynucleotide expression can be detected in a number of different ways. As described *infra*, the expression level of a polynucleotide of the invention in a cell can be determined by probing the mRNA expressed in a cell with a probe that specifically hybridizes with a transcript (or complementary nucleic acid derived therefrom) of a polynucleotide of the invention. Probing can be conducted by lysing the cells and conducting Northern blots or without lysing the cells using in situ-hybridization techniques. Alternatively, a polypeptide of the invention can be detected using immunological methods in which a cell lysate is probed with antibodies that specifically bind to a polypeptide of the invention.

[0206] Other cell-based assays are reporter assays conducted with cells that do not express a polypeptide or polynucleotide of the invention. Certain of these assays are conducted with

a heterologous nucleic acid construct that includes a promoter of a polynucleotide of the invention that is operably linked to a reporter gene that encodes a detectable product. A number of different reporter genes can be utilized. Some reporters are inherently detectable. An example of such a reporter is green fluorescent protein that emits fluorescence that can be detected with a fluorescence detector. Other reporters generate a detectable product. Often such reporters are enzymes. Exemplary enzyme reporters include, but are not limited to, β-glucuronidase, chloramphenicol acetyl transferase (CAT); Alton and Vapnek (1979) *Nature* 282:864-869), luciferase, β-galactosidase, green fluorescent protein (GFP) and alkaline phosphatase (Toh, *et al.* (1980) *Eur. J. Biochem.* 182:231-238; and Hall *et al.* (1983) *J. Mol. Appl. Gen.* 2:101).

[0207] In these assays, cells harboring the reporter construct are contacted with a test compound. A test compound that either activates the promoter by binding to it or triggers a cascade that produces a molecule that activates the promoter causes expression of the detectable reporter. Certain other reporter assays are conducted with cells that harbor a heterologous construct that includes a transcriptional control element that activates expression of a polynucleotide of the invention and a reporter operably linked thereto. Here, too, an agent that binds to the transcriptional control element to activate expression of the reporter or that triggers the formation of an agent that binds to the transcriptional control element to activate reporter expression, can be identified by the generation of signal associated with reporter expression.

[0208] The level of expression or activity can be compared to a baseline value. As indicated above, the baseline value can be a value for a control sample or a statistical value that is representative of expression levels for a control population (*e.g.*, healthy individuals not having or at risk for mood disorders or psychotic disorders). Expression levels can also be determined for cells that do not express a polynucleotide of the invention as a negative control. Such cells generally are otherwise substantially genetically the same as the test cells.

[0209] A variety of different types of cells can be utilized in the reporter assays. Cells that express an endogenous polypeptide or polynucleotide of the invention include, *e.g.*, brain cells, including cells from the cerebellum, anterior cingulate cortex, dorsolateral prefrontal cortex, amygdala, hippocampus, or nucleus accumbens. Cells that do not endogenously express polynucleotides of the invention can be prokaryotic, but are preferably eukaryotic. The eukaryotic cells can be any of the cells typically utilized in generating cells that harbor

recombinant nucleic acid constructs. Exemplary eukaryotic cells include, but are not limited to, yeast, and various higher eukaryotic cells such as the COS, CHO and HeLa cell lines.

[0210] Various controls can be conducted to ensure that an observed activity is authentic including running parallel reactions with cells that lack the reporter construct or by not contacting a cell harboring the reporter construct with test compound. Compounds can also be further validated as described below.

3. Catalytic activity

[0211] Catalytic activity of polypeptides of the invention 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).

[0212] Analysis of the activity of polypeptides of the invention 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.

4. Validation

[0213] 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 of the invention 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, primates, mice, and rats. As described herein, models using admininstration of known therapeutics can be useful.

5. Animal models

[0214] Animal models of mental disorders also find use in screening for modulators. In one embodiment, invertebrate models such as *Drosophila* models can be used, screening for modulators of *Drosophila* orthologs of the human genes disclosed herein. In another

embodiment, 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 of the invention. The same technology can also be applied to make knockout cells. When desired, tissue-specific expression or knockout of a polynucleotide or polypeptide of the invention 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.

[0215] 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 of the invention with a mutated version of the polynucleotide, or by mutating an endogenous polynucleotide, *e.g.*, by exposure to carcinogens.

[0216] 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 Laboratory (1988) and *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, ed., IRL Press, Washington, D.C., (1987).

B. Modulators of Polypeptides or Polynucleotides of the Invention

[0217] The agents tested as modulators of the polypeptides or polynucleotides of the invention can be any small chemical compound, or a biological entity, such as a protein, sugar, nucleic acid or lipid. Alternatively, modulators can be genetically altered versions of a polypeptide or polynucleotides, *e.g.,* recombinant or altered versions of FGF9, FGF2, NCAM, or a peptide inhibitor of the FGF system. Typically, test compounds will be small chemical molecules and peptides. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds

that can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (*e.g.*, in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland) and the like. Modulators also include agents designed to reduce the level of mRNA of the invention (*e.g.* antisense molecules, ribozymes, DNAzymes and the like) or the level of translation from an mRNA.

[0218] In one preferred embodiment, 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 (particular chemical species or subclasses) 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.

[0219] 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.

[0220] 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).

[0221] 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

[0222] In the high throughput assays of the invention, 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 of the invention. More recently, microfluidic approaches to reagent manipulation have been developed.

[0223] 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.

[0224] 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).

[0225] 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.

[0226] 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.

[0227] 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.

[0228] 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 of various 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.

[0229] The invention provides *in vitro* assays for identifying, in a high throughput format, compounds that can modulate the expression or activity of the polynucleotides or polypeptides of the invention. In a preferred embodiment, the methods of the invention include such 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.

[0230] 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 of the invention 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 of the invention can be added, and the resulting decrease in signal for the expression or activity can be similarly detected.

D. Computer-Based Assays

[0231] Yet another assay for compounds that modulate the activity of a polypeptide or polynucleotide of the invention 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 analyses can be performed on potential receptors or binding partners of the polypeptides or polynucleotides of the invention. 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 of the invention. These regions are then used to identify polypeptides that bind to a polypeptide or polynucleotide of the invention.

[0232] 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.

[0233] 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.

[0234] 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.

[0235] 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 of the invention. 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.

[0236] Computer systems are also used to screen for mutations, polymorphic variants, alleles and interspecies homologs of genes encoding a polypeptide or polynucleotide of the invention. 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 homologs. 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 of the invention involves

receiving input of a first amino acid sequence of a polypeptide of the invention (or of a first nucleic acid sequence encoding a polypeptide of the invention), *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 of the invention, and mutations associated with disease states and genetic traits.

VIII. Compositions, Kits and Integrated Systems

[0237] The invention provides compositions, kits and integrated systems for practicing the assays described herein using polypeptides or polynucleotides of the invention, antibodies specific for polypeptides or polynucleotides of the invention, *etc.*

[0238] The invention provides assay compositions for use in solid phase assays; such compositions can include, for example, one or more polynucleotides or polypeptides of the invention immobilized on a solid support, and a labeling reagent. For example, the kit could include an array consisting of a set or subset of the informative sequences listed in FIGURE 1, FIGURES 5-8, or Tables 1-4. In each case, the assay compositions can also include additional reagents that are desirable for hybridization. Modulators of expression or activity of polynucleotides or polypeptides of the invention can also be included in the assay compositions.

[0239] The invention also provides kits for carrying out the therapeutic and diagnostic assays of the invention. The kits typically include a probe that comprises an antibody that specifically binds to polypeptides or polynucleotides of the invention, and a label for detecting the presence of the probe. The kits may include several polynucleotide sequences encoding polypeptides of the invention. Kits can include any of the compositions noted above, and optionally further include additional components such as instructions to practice a high-throughput method of assaying for an effect on expression of the genes encoding the polypeptides of the invention, or on activity of the polypeptides of the invention, one or more

containers or compartments (*e.g.*, to hold the probe, labels, or the like), a control modulator of the expression or activity of polypeptides of the invention, a robotic armature for mixing kit components or the like.

[0240] The invention also provides integrated systems for high-throughput screening of potential modulators for an effect on the expression or activity of the polypeptides of the invention. The systems typically include a robotic armature which transfers fluid from a source to a destination, a controller which controls the robotic armature, a label detector, a data storage unit which records label detection, and an assay component such as a microtiter dish comprising a well having a reaction mixture or a substrate comprising a fixed nucleic acid or immobilization moiety.

[0241] A number of robotic fluid transfer systems are available, or can easily be made from existing components. For example, a Zymate XP (Zymark Corporation; Hopkinton, MA) automated robot using a Microlab 2200 (Hamilton; Reno, NV) pipetting station can be used to transfer parallel samples to 96 well microtiter plates to set up several parallel simultaneous STAT binding assays.

[0242] Optical images viewed (and, optionally, recorded) by a camera or other recording device (*e.g.*, a photodiode and data storage device) are optionally further processed in any of the embodiments herein, *e.g.*, by digitizing the image and storing and analyzing the image on a computer. A variety of commercially available peripheral equipment and software is available for digitizing, storing and analyzing a digitized video or digitized optical image, *e.g.*, using PC (Intel x86 or Pentium chip-compatible DOS®, OS2® WINDOWS®, WINDOWS NT®, WINDOWS95®, WINDOWS98®, or WINDOWS2000® based computers), MACINTOSH®, or UNIX® based (*e.g.*, SUN® work station) computers.

[0243] One conventional system carries light from the specimen field to a cooled charge-coupled device (CCD) camera, in common use in the art. A CCD camera includes an array of picture elements (pixels). The light from the specimen is imaged on the CCD. Particular pixels corresponding to regions of the specimen (*e.g.*, individual hybridization sites on an array of biological polymers) are sampled to obtain light intensity readings for each position. Multiple pixels are processed in parallel to increase speed. The apparatus and methods of the invention are easily used for viewing any sample, *e.g.*, by fluorescent or dark field microscopic techniques.

IX. Administration and Pharmaceutical Compositions

[0244] Modulators of the polynucleotides or polypeptides of the invention (*e.g.*, antagonists or agonists, such as FGF9, 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.

[0245] 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 Exacerbation (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, various anxiety disorders, and substance-induced psychotic disorder.

[0246] In some embodiments, modulators of polynucleotides or polypeptides of the invention can be combined with other drugs useful for treating mental disorders including useful for treating mood disorders, *e.g.*, schizophrenia, bipolar disorders, major depression, and/or anxiety disorders. In some preferred embodiments, pharmaceutical compositions of the invention comprise a modulator of a polypeptide of polynucleotide of the invention 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. In other embodiments, modulators or polypeptides of the invention, in particular FGF2, can be used to treat and/or prevent learning disabilities, memory loss, or disorders associated with learning disability and memory loss. In still other embodiments, FGF9 can used to treat anxiety disorders and symptoms relating to anxiety that may be associated with the above-mentioned disorders (*e.g.,* major depression disorder).

[0247] 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)).

[0248] The modulators (*e.g.*, agonists or antagonists) of the expression or activity of the a polypeptide or polynucleotide of the invention, 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.

[0249] 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.

[0250] 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.

[0251] 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 subject.

[0252] 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.

X. Gene Therapy Applications

[0253] A variety of human diseases can be treated by therapeutic approaches that involve stably introducing a gene into a human cell such that the gene is transcribed and the gene

product is produced in the cell. Diseases amenable to treatment by this approach include inherited diseases, including those in which the defect is in a single or multiple genes. Gene therapy is also useful for treatment of acquired diseases and other conditions. For discussions on the application of gene therapy towards the treatment of genetic as well as acquired diseases, *see*, Miller, *Nature* 357:455-460 (1992); and Mulligan, *Science* 260:926-932 (1993).

[0254] In the context of the present invention, gene therapy can be used for treating a variety of disorders and/or diseases in which the polynucleotides and polypeptides of the invention has been implicated. For example, compounds, including polynucleotides, can be identified by the methods of the present invention as effective in treating a mental disorder. Introduction by gene therapy of these polynucleotides can then be used to treat, *e.g.*, mental disorders including mood disorders and psychotic disorders.

A. Vectors for Gene Delivery

[0255] For delivery to a cell or organism, the polynucleotides of the invention can be incorporated into a vector. Examples of vectors used for such purposes include expression plasmids capable of directing the expression of the nucleic acids in the target cell. In other instances, the vector is a viral vector system wherein the nucleic acids are incorporated into a viral genome that is capable of transfecting the target cell. In a preferred embodiment, the polynucleotides can be operably linked to expression and control sequences that can direct expression of the gene in the desired target host cells. Thus, one can achieve expression of the nucleic acid under appropriate conditions in the target cell.

B. Gene Delivery Systems

[0256] Viral vector systems useful in the expression of the nucleic acids include, for example, naturally occurring or recombinant viral vector systems. Depending upon the particular application, suitable viral vectors include replication competent, replication deficient, and conditionally replicating viral vectors. For example, viral vectors can be derived from the genome of human or bovine adenoviruses, vaccinia virus, herpes virus, adeno-associated virus, minute virus of mice (MVM), HIV, sindbis virus, and retroviruses (including but not limited to Rous sarcoma virus), and MoMLV. Typically, the genes of interest are inserted into such vectors to allow packaging of the gene construct, typically with accompanying viral DNA, followed by infection of a sensitive host cell and expression of the gene of interest.

[0257] As used herein, "gene delivery system" refers to any means for the delivery of a nucleic acid of the invention to a target cell. In some embodiments of the invention, nucleic acids are conjugated to a cell receptor ligand for facilitated uptake (*e.g.*, invagination of coated pits and internalization of the endosome) through an appropriate linking moiety, such as a DNA linking moiety (Wu *et al.*, *J. Biol. Chem.* 263:14621-14624 (1988); WO 92/06180). For example, nucleic acids can be linked through a polylysine moiety to asialo-oromucocid, which is a ligand for the asialoglycoprotein receptor of hepatocytes.

[0258] Similarly, viral envelopes used for packaging gene constructs that include the nucleic acids of the invention can be modified by the addition of receptor ligands or antibodies specific for a receptor to permit receptor-mediated endocytosis into specific cells (*see, e.g.*, WO 93/20221, WO 93/14188, and WO 94/06923). In some embodiments of the invention, the DNA constructs of the invention are linked to viral proteins, such as adenovirus particles, to facilitate endocytosis (Curiel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88:8850-8854 (1991)). In other embodiments, molecular conjugates of the instant invention can include microtubule inhibitors (WO/9406922), synthetic peptides mimicking influenza virus hemagglutinin (Plank *et al.*, *J. Biol. Chem.* 269:12918-12924 (1994)), and nuclear localization signals such as SV40 T antigen (WO93/19768).

[0259] Retroviral vectors are also useful for introducing the nucleic acids of the invention into target cells or organisms. Retroviral vectors are produced by genetically manipulating retroviruses. The viral genome of retroviruses is RNA. Upon infection, this genomic RNA is reverse transcribed into a DNA copy which is integrated into the chromosomal DNA of transduced cells with a high degree of stability and efficiency. The integrated DNA copy is referred to as a provirus and is inherited by daughter cells as is any other gene. The wild type retroviral genome and the proviral DNA have three genes: the *gag*, the *pol* and the *env* genes, which are flanked by two long terminal repeat (LTR) sequences. The *gag* gene encodes the internal structural (nucleocapsid) proteins; the *pol* gene encodes the RNA directed DNA polymerase (reverse transcriptase); and the *env* gene encodes viral envelope glycoproteins. The 5' and 3' LTRs serve to promote transcription and polyadenylation of virion RNAs. Adjacent to the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient encapsulation of viral RNA into particles (the Psi site) (*see*, Mulligan, In: *Experimental Manipulation of Gene Expression*,

Inouye (ed), 155-173 (1983); Mann *et al.*, *Cell* 33:153-159 (1983); Cone and Mulligan, *Proceedings of the National Academy of Sciences, U.S.A.*, 81:6349-6353 (1984)).

[0260] The design of retroviral vectors is well known to those of ordinary skill in the art. In brief, if the sequences necessary for encapsidation (or packaging of retroviral RNA into infectious virions) are missing from the viral genome, the result is a *cis*-acting defect which prevents encapsidation of genomic RNA. However, the resulting mutant is still capable of directing the synthesis of all virion proteins. Retroviral genomes from which these sequences have been deleted, as well as cell lines containing the mutant genome stably integrated into the chromosome are well known in the art and are used to construct retroviral vectors. Preparation of retroviral vectors and their uses are described in many publications including, *e.g.*, European Patent Application EPA 0 178 220; U.S. Patent 4,405,712, Gilboa *Biotechniques* 4:504-512 (1986); Mann *et al.*, *Cell* 33:153-159 (1983); Cone and Mulligan *Proc. Natl. Acad. Sci. USA* 81:6349-6353 (1984); Eglitis *et al. Biotechniques* 6:608-614 (1988); Miller *et al. Biotechniques* 7:981-990 (1989); Miller (1992) *supra*; Mulligan (1993), *supra*; and WO 92/07943.

[0261] The retroviral vector particles are prepared by recombinantly inserting the desired nucleotide sequence into a retrovirus vector and packaging the vector with retroviral capsid proteins by use of a packaging cell line. The resultant retroviral vector particle is incapable of replication in the host cell but is capable of integrating into the host cell genome as a proviral sequence containing the desired nucleotide sequence. As a result, the patient is capable of producing, for example, a polypeptide or polynucleotide of the invention and thus restore the cells to a normal phenotype.

[0262] Packaging cell lines that are used to prepare the retroviral vector particles are typically recombinant mammalian tissue culture cell lines that produce the necessary viral structural proteins required for packaging, but which are incapable of producing infectious virions. The defective retroviral vectors that are used, on the other hand, lack these structural genes but encode the remaining proteins necessary for packaging. To prepare a packaging cell line, one can construct an infectious clone of a desired retrovirus in which the packaging site has been deleted. Cells comprising this construct will express all structural viral proteins, but the introduced DNA will be incapable of being packaged. Alternatively, packaging cell lines can be produced by transforming a cell line with one or more expression plasmids

encoding the appropriate core and envelope proteins. In these cells, the *gag*, *pol*, and *env* genes can be derived from the same or different retroviruses.

[0263] A number of packaging cell lines suitable for the present invention are also available in the prior art. Examples of these cell lines include Crip, GPE86, PA317 and PG13 (*see* Miller *et al.*, *J. Virol.* 65:2220-2224 (1991)). Examples of other packaging cell lines are described in Cone and Mulligan *Proceedings of the National Academy of Sciences, USA,* 81:6349-6353 (1984); Danos and Mulligan *Proceedings of the National Academy of Sciences, USA,* 85:6460-6464 (1988); Eglitis *et al.* (1988), *supra*; and Miller (1990), *supra*.

[0264] Packaging cell lines capable of producing retroviral vector particles with chimeric envelope proteins may be used. Alternatively, amphotropic or xenotropic envelope proteins, such as those produced by PA317 and GPX packaging cell lines may be used to package the retroviral vectors.

[0265] In some embodiments of the invention, an antisense polynucleotide is administered which hybridizes to a gene encoding a polypeptide of the invention. The antisense polypeptide can be provided as an antisense oligonucleotide (*see, e.g.*, Murayama *et al.*, *Antisense Nucleic Acid Drug Dev.* 7:109-114 (1997)). Genes encoding an antisense nucleic acid can also be provided; such genes can be introduced into cells by methods known to those of skill in the art. For example, one can introduce an antisense nucleotide sequence in a viral vector, such as, for example, in hepatitis B virus (*see, e.g.*, Ji *et al.*, *J. Viral Hepat.* 4:167-173 (1997)), in adeno-associated virus (*see, e.g.*, Xiao *et al.*, *Brain Res.* 756:76-83 (1997)), or in other systems including, but not limited, to an HVJ (Sendai virus)-liposome gene delivery system (*see, e.g.*, Kaneda *et al.*, *Ann. NY Acad. Sci.* 811:299-308 (1997)), a "peptide vector" (*see, e.g.*, Vidal *et al.*, *CR Acad. Sci III* 32:279-287 (1997)), as a gene in an episomal or plasmid vector (*see, e.g.*, Cooper *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:6450-6455 (1997), Yew *et al. Hum Gene Ther.* 8:575-584 (1997)), as a gene in a peptide-DNA aggregate (*see, e.g.*, Niidome *et al.*, *J. Biol. Chem.* 272:15307-15312 (1997)), as "naked DNA" (*see, e.g.*, U.S. patent Nos. 5,580,859 and 5,589,466), in lipidic vector systems (*see, e.g.*, Lee *et al.*, *Crit Rev Ther Drug Carrier Syst.* 14:173-206 (1997)), polymer coated liposomes (U.S. patent Nos. 5,213,804 and 5,013,556), cationic liposomes (Epand *et al.*, U.S. patent Nos. 5,283,185; 5,578,475; 5,279,833; and 5,334,761), gas filled microspheres (U.S. patent No. 5,542,935), ligand-targeted encapsulated macromolecules (U.S. patent Nos. 5,108,921; 5,521,291; 5,554,386; and 5,166,320).

[0266] Upregulated transcripts listed in the biomarker tables herein which are correlated with mental disorders may be targeted with one or more short interfering RNA (siRNA) sequences that hybridize to specific sequences in the target, as described above. Targeting of certain brain transcripts with siRNA in vivo has been reported, for example, by Zhang *et al.*, *J. Gene. Med.*, 12:1039-45 (2003), who utilized monoclonal antibodies against the transferrin receptor to facilitate passage of liposome-encapsulated siRNA molecules through the blood brain barrier. Targeted siRNAs represent useful therapeutic compounds for attenuating the over-expressed transcripts that are associated with disease states, *e.g.*, MDD, BP, and other mental disorders.

[0267] In another embodiment, conditional expression systems, such as those typified by the tet-regulated systems and the RU-486 system, can be used (see, *e.g.*, Gossen & Bujard, *PNAS* 89:5547 (1992); Oligino et al., Gene Ther. 5:491-496 (1998); Wang *et al.*, *Gene Ther.* 4:432-441 (1997); Neering et al., *Blood* 88:1147-1155 (1996); and Rendahl et al., *Nat. Biotechnol.* 16:757-761 (1998)). These systems impart small molecule control on the expression of the target gene(s) of interest.

[0268] In another embodiment, stem cells engineered to express a transcript of interest can implanted into the brain.

C. Pharmaceutical Formulations

[0269] When used for pharmaceutical purposes, the vectors used for gene therapy are formulated in a suitable buffer, which can be any pharmaceutically acceptable buffer, such as phosphate buffered saline or sodium phosphate/sodium sulfate, Tris buffer, glycine buffer, sterile water, and other buffers known to the ordinarily skilled artisan such as those described by Good *et al. Biochemistry* 5:467 (1966).

[0270] The compositions can additionally include a stabilizer, enhancer, or other pharmaceutically acceptable carriers or vehicles. A pharmaceutically acceptable carrier can contain a physiologically acceptable compound that acts, for example, to stabilize the nucleic acids of the invention and any associated vector. A physiologically acceptable compound can include, for example, carbohydrates, such as glucose, sucrose or dextrans; antioxidants, such as ascorbic acid or glutathione; chelating agents; low molecular weight proteins or other stabilizers or excipients. Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents, or preservatives, which are particularly useful

for preventing the growth or action of microorganisms. Various preservatives are well known and include, for example, phenol and ascorbic acid. Examples of carriers, stabilizers, or adjuvants can be found in Remington's *Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985).

D. Administration of Formulations

[0271] The formulations of the invention can be delivered to any tissue or organ using any delivery method known to the ordinarily skilled artisan. In some embodiments of the invention, the nucleic acids of the invention are formulated in mucosal, topical, and/or buccal formulations, particularly mucoadhesive gel and topical gel formulations. Exemplary permeation enhancing compositions, polymer matrices, and mucoadhesive gel preparations for transdermal delivery are disclosed in U.S. Patent No. 5,346,701.

E. Methods of Treatment

[0272] The gene therapy formulations of the invention are typically administered to a cell. The cell can be provided as part of a tissue, such as an epithelial membrane, or as an isolated cell, such as in tissue culture. The cell can be provided *in vivo*, *ex vivo*, or *in vitro*.

[0273] The formulations can be introduced into the tissue of interest *in vivo* or *ex vivo* by a variety of methods. In some embodiments of the invention, the nucleic acids of the invention are introduced into cells by such methods as microinjection, calcium phosphate precipitation, liposome fusion, or biolistics. In further embodiments, the nucleic acids are taken up directly by the tissue of interest.

[0274] In some embodiments of the invention, the nucleic acids of the invention are administered *ex vivo* to cells or tissues explanted from a patient, then returned to the patient. Examples of *ex vivo* administration of therapeutic gene constructs include Nolta *et al.*, *Proc Natl. Acad. Sci. USA* 93(6):2414-9 (1996); Koc *et al.*, *Seminars in Oncology* 23 (1):46-65 (1996); Raper *et al.*, *Annals of Surgery* 223(2):116-26 (1996); Dalesandro *et al.*, *J. Thorac. Cardi. Surg.*, 11(2):416-22 (1996); and Makarov *et al.*, *Proc. Natl. Acad. Sci. USA* 93(1):402-6 (1996).

XI. Diagnosis of Mood Disorders and Psychotic Disorders

[0275] The present invention also provides methods of diagnosing mood disorders (such as major depression or bipolar disorder), psychotic disorders (such as schizophrenia), or a

predisposition of at least some of the pathologies of such disorders. Diagnosis involves determining the level of a polypeptide or polynucleotide of the invention in a patient and then comparing the level to a baseline or range. Typically, the baseline value is representative of a polypeptide or polynucleotide of the invention in a healthy person not suffering from a mood disorder or a psychotic disorder or under the effects of medication or other drugs. Variation of levels of a polypeptide or polynucleotide of the invention from the baseline range (either up or down) indicates that the patient has a mood disorder or a psychotic disorder or at risk of developing at least some aspects of a mood disorder or a psychotic disorder. In some embodiments, the level of a polypeptide or polynucleotide of the invention are measured by taking a blood, urine or tissue sample from a patient and measuring the amount of a polypeptide or polynucleotide of the invention in the sample using any number of detection methods, such as those discussed herein.

[0276] Antibodies can be used in assays to detect differential protein expression in patient samples, *e.g.*, ELISA assays, immunoprecipitation assays, and immunohistochemical assays. PCR assays can be used to detect expression levels of nucleic acids, as well as to discriminate between variants in genomic structure or transcription, such as the FGFR splice variants shown in Figure 1 and described in the Examples.

[0277] In the case where absence of gene expression is associated with a disorder, the genomic structure of a gene can be evaluated with known methods such as PCR to detect deletion or insertion mutations associated with disease suspectibility. Conversely, the presence of mRNA or protein corresponding to a particular gene would indicate that an individual does not have the genetic mutation associated with the lack of gene expression or the associated disorder. Thus, diagnosis can be made by detecting the presence or absence of mRNA or protein, or by examining the genomic structure of the gene.

[0278] Single nucleotide polymorphism (SNP) analysis is also useful for detecting differences between alleles of the polynucleotides (*e.g.*, genes) of the invention. SNPs linked to genes encoding polypeptides of the invention are useful, for instance, for diagnosis of diseases (*e.g.*, mood disorders such as bipolar disease, major depression, and schizophrenia disorders) whose occurrence is linked to the gene sequences of the invention. For example, if an individual carries at least one SNP linked to a disease-associated allele of the gene sequences of the invention, the individual is likely predisposed for one or more of those diseases. If the individual is homozygous for a disease-linked SNP, the individual is

particularly predisposed for occurrence of that disease. In some embodiments, the SNP associated with the gene sequences of the invention is located within 300,000; 200,000; 100,000; 75,000; 50,000; or 10,000 base pairs from the gene sequence.

[0279] Various real-time PCR methods can be used to detect SNPs, including, *e.g.*, Taqman or molecular beacon-based assays (*e.g.*, U.S. Patent Nos. 5,210,015; 5,487,972; Tyagi *et al.*, *Nature Biotechnology* 14:303 (1996); and PCT WO 95/13399 are useful to monitor for the presence of absence of a SNP. Additional SNP detection methods include, *e.g.*, DNA sequencing, sequencing by hybridization, dot blotting, oligonucleotide array (DNA Chip) hybridization analysis, or are described in, *e.g.*, U.S. Patent No. 6,177,249; Landegren *et al.*, *Genome Research*, 8:769-776 (1998); Botstein *et al.*, *Am J Human Genetics* 32:314-331 (1980); Meyers *et al.*, Methods in Enzymology 155:501-527 (1987); Keen *et al.*, *Trends in Genetics* 7:5 (1991); Myers *et al.*, *Science* 230:1242-1246 (1985); and Kwok *et al.*, *Genomics* 23:138-144 (1994). PCR methods can also be used to detect deletion/insertion polymorphisms, such as the deletion polymorphism of the PSPHL gene associated with suspectibility to BP.

[0280] In some embodiments, the level of the enzymatic product of a polypeptide or polynucleotide of the invention is measured and compared to a baseline value of a healthy person or persons. Modulated levels of the product compared to the baseline indicates that the patient has a mood disorder or a psychotic disorder or is at risk of developing at least some aspects of a mood disorder or a psychotic disorder. Patient samples, for example, can be blood, urine or tissue samples. In some cases, one skilled in the art could use expression of genes in readily obtainable cells, *e.g.*, lymphocytes, as a proxy for evaluation expression of those genes in one or more regions of the brain.

XII. Examples

[0281] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Differential Expression of Genes Associated with Suicide in Both BP and MDD Subjects

[0282] 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.

[0283] 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.

[0284] 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.

[0285] 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

[0286] 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

[0287] 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. 20040152111-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.

[0288] **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.

[0289] 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.

[0290] 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.

[0291] **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

[0292] 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)

[0293] 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

[0294] 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

[0295] **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 candidate approach and a gene discovery approach with laser-capture microdissection of the dentate gyrus followed by microarray analyses.

[0296] **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 Vgf was increased, while expression of NCAM was decreased.

Example 5: Anxiolytic Effect of Chronically Administered FGF2

[0297] 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.

[0298] 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.

[0299] 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.

[0300] 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).

[0301] 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.

[0302] 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 Stress

[0303] 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.

[0304] 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.

[0305] 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.

[0306] Forced swim testing. To test for possible FGF2 injection effects on anti-depressant 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.

[0307] 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.

[0308] 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.

[0309] 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.

[0310] 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).

[0311] 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).

[0312] 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.

[0313] 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.

[0314] 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

[0315] 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.

[0316] 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.

[0317] 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.

[0318] Genes that are almost exclusively expressed in glia are significantly downregulated. These genes are useful markers for global glial alterations in MDD patients.

[0319] 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.

Example 8. Anxiolytic Effect of FGF9

[0320] Rats were implanted with guide cannulae into the left ventricle. Adult male Sprague-Dawley rats were microinjected (intracerebroventricularly (icv)) with either vehicle or FGF9 at the concentrations indicated in FIGURE 13. Total volumes (8 μl) were infused at 1.0 μl/min and an injection syringe was held in place for 3 min to allow for diffusion. Fifteen minutes after microinjection, anxiety was tested in the rats using the elevated plus maze ("EPM"). Rats were placed in the center of the EPM and allowed to explore freely under dim light for 5 min. The animals were evaluated for time spent in the center, open arms, and

closed arms of the maze, where increased time in the open arms versus the closed arm correlates with less anxiety. Twenty-four hours after microinjection, depression was evaluated in the rats using the forced swim test (FST).

[0321] The data show that acute administration of FGF9 at 2 to 2000 ng successfully decreased anxiety-related behavior using this animal model system.

Example 9. Effect of Chronic Administration of FGF9

[0322] Animals were injected icv daily for 3 weeks with 20 ng FGF9. All behavioral testing was performed in the lights on portion of the light/dark cycle. FIGURE 14 shows that chronic FGF9 administration increased the depression-like effect seen in the forced swim test compared to acute administration. Chronic FGF9 had an anxiogenic effect on the elevated plus maze task, which is opposite to the anxiolytic effect seen with acute administration.

Example 10. Effect of siRNAs Targeting FGF 2 or FGF9

[0323] Small interfering RNAs (siRNAs) targeting either FGF2 or FGF9 expression were designed and validated. COS7 cells were transfected with a plasmid vector (psiCHECK-2, Promega) containing either FGF2 or FGF9 and co-transfected with either FGF2 or FGF9 siRNA. The transfection efficiency and the ability of siRNA targeting FGF2 or FGF9 to decrease protein levels were evaluated by a dual-luciferase reporter assay. The effects of FGF2 siRNA inhibition on FGF2 expression was determined in a human gliomal cell line (U87MG) that endogenously expresses high levels of FGF2. Sequences have been identified that are effective (>80% knockdown) and specific *in vitro*.

siRNA	Sense Strand (5' → 3')	Antisense Strand (5' → 3')
FGF9	UGGAUGAGGACUUGCCGAUUU	AUCGGCAAGUCCUCAUCCAUU

The "UU" nucleotides at the 3' end of each strand correspond to 3' overhangs, but the sense and/or antisense strand may alternatively comprise "dTdT" 3' overhangs or 3' overhangs that have complementarity to the target sequence or the complementary strand thereof.

[0324] All publications, databases, Genbank sequences, patents, and patent applications cited herein are hereby incorporated by reference.

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
CAPN3	2 region	"Calpain 3, (p94)"	BC003169	15q15.1-q21.1	0.783	0.799	0.567	0.651	0.846	0.926
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
CNTN1	ancng	Contactin 1	U07819	12q11-q12	1.320	1.283	1.038	1.098	0.883	0.994

TABLE 1A (cont'd)

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
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-galactose ceramide galactosyltransferase)	CN275924	4q26	0.945	0.781	0.710	0.776	0.926	0.947
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

TABLE 1A (cont'd)

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
ENPP2	amygdala	Ectonucleotide pyrophosphatase/phosphodiestera se 2 (autotaxin)	CR606785	8q24.1	0.816	0.817	0.602	0.717	0.813	0.851
ENPP2	amygdala	Ectonucleotide pyrophosphatase/phosphodiestera se 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	amygdala	V-fos FBJ murine osteosarcoma viral oncogene homolog	BM894421	14q24.3	0.671	0.679	0.716	0.638	0.770	0.659
CNP	amygdala	"2',3'-cyclic nucleotide 3' phosphodiesterase"	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

TABLE 1A (cont'd)

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
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	amygdala	Fatty acid 2-hydroxylase	AA454978	16q23	0.898	0.875	0.685	0.734	0.906	0.922
C20orf35	amygdala	Chromosome 20 open reading frame 35	BI711408	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	0.828	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)	AI741795	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
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		

TABLE 1A (cont'd)

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
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"	AI554919	6p21.3	0.874	0.860	0.831	0.839	0.771	0.799

TABLE 1B

Symbol	Suicide dysregulation	Name	Best Accession	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 synaptic membrane exocytosis 2	BC043144	8q22.3	1.243	1.288
TM4SF9	ancng	Tetraspanin 5	BX649011	4q23	1.488	1.358
PCLO	ancng	Piccolo (presynaptic cytomatrix protein)	AB011131	7q11.23-q21.3	1.555	1.383
GNB5	ancng	Guanine nucleotide binding protein (G protein), beta 5	AK092059	15q21.2	1.206	1.263
GABRA1	ancng	Gamma-aminobutyric acid (GABA) A receptor, 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
R3HDM	ancng	R3H domain containing 1	BX538168	2q21.3	1.208	1.222
COL5A2	ancng	Collagen, type V, alpha 2	NM_000393	2q14-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

TABLE 1B (cont'd)

Symbol	Suicide dysregulation	Name	Best Accession	Cytoband	Anterior Cingulate Fold Change	
					Suicidal MDD Vs Non-suicidal MDD	Suicidal MDD Vs Control
CADPS2	ancng	Ca2+-dependent 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 histocompatibility complex, class I, A	AB209117	6p21.3	0.771	0.788
LYPDC1	ancng	LY6/PLAUR domain containing 1	AK122643	2q21.2	0.819	0.787
RYR1	ancng	Ryanodine receptor 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
CDH18	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 polypeptide 50	BC000272	10q22.1	1.338	1.219
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 histocompatibility 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
SST	Hs.12409	3q28	Somatostatin
UBQLN2	Hs.522668	Xp11.23-p11.1	Ubiquilin 2
UXT	Hs.172791	Xp11.23-p11.22	Ubiquitously-expressed transcript

SUBSTITUTE SHEET (RULE 26)

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
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, homolog (Xenopus laevis) (GREM1), mRNA	-1.153	0.020	-1.099	0.194
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 (VEGFC), 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
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

TABLE 4 (cont'd)

BPD genes					
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
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 convertase) (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

WHAT IS CLAIMED IS:

1. A method for alleviating one or more symptoms of anxiety in a mammalian subject, said method comprising administering an effective amount of FGF9 to said subject.

2. The method of claim 1, wherein said effective amount of FGF9 is administered to said subject by injection.

3. The method of claim 1, wherein said effective amount of FGF9 is acutely administered to said subject.

4. The method of claim 1, wherein said subject has been diagnosed with an anxiety disorder.

5. The method of claim 1, wherein said subject has been diagnosed with major depression disorder.

6. The method of claim 1, wherein said subject is a human.

7. A method for reducing risk-taking behavior in a mammalian subject, said method comprising administering an effective amount of FGF9 to said subject.

8. The method of claim 7, wherein said effective amount of FGF9 is administered to said subject by injection.

9. The method of claim 7, wherein said effective amount of FGF9 is chronically administered to said subject.

10. The method of claim 7, wherein said subject has high FGF2 levels.

11. The method of claim 7, wherein said subject is a human.

Fig. 1 (Sheet 1)

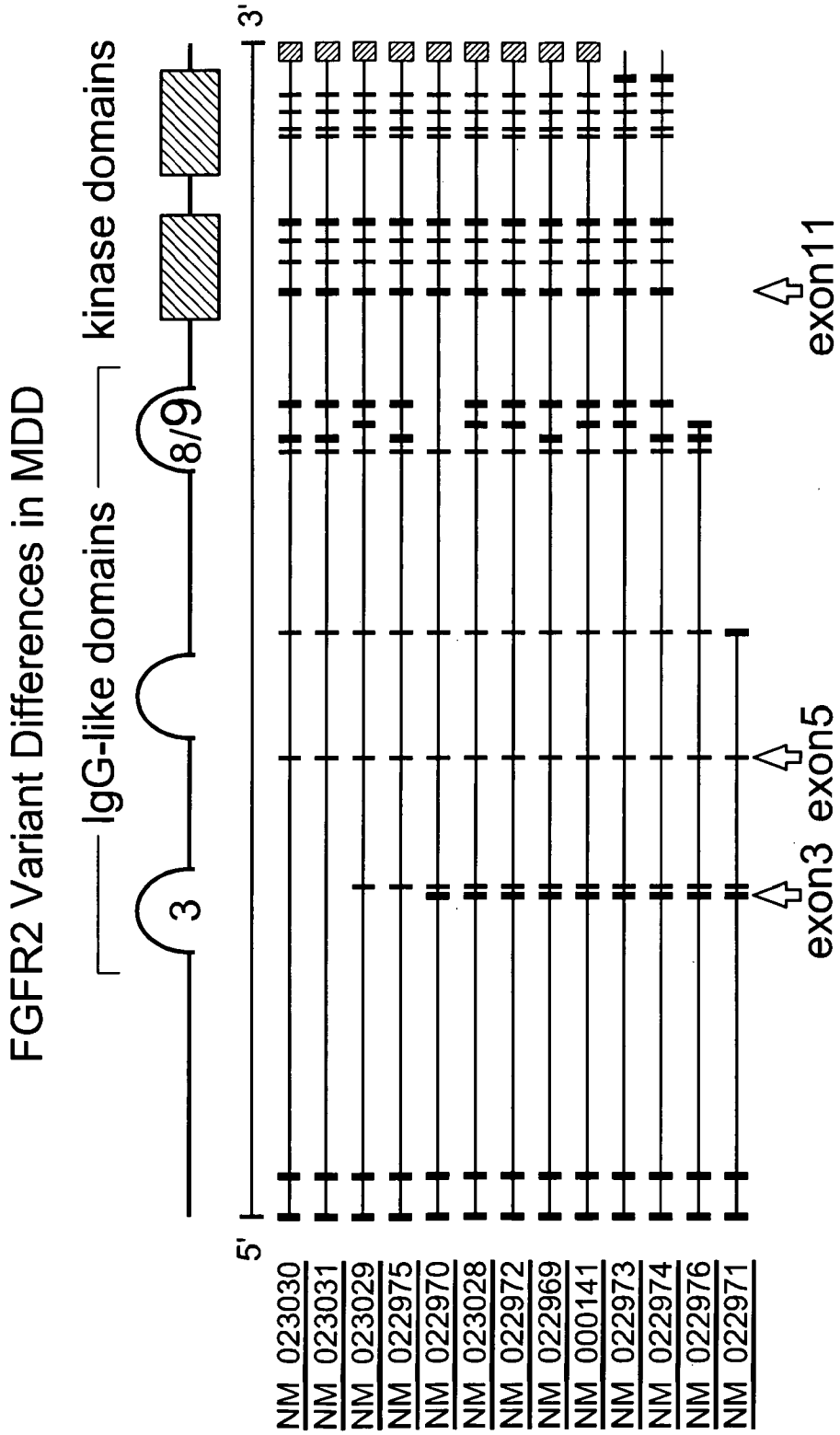

FGFR2 Variant Differences in MDD
IgG-like domains
kinase domains
3
8/9
5'
3'
exon3
exon5
exon11
NM_023030
NM_023031
NM_023029
NM_022975
NM_022970
NM_023028
NM_022972
NM_022969
NM_000141
NM_022973
NM_022974
NM_022976
NM_022971

Fig. 1 (Sheet 2)

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. 2

FST (top) and EPM (bottom)

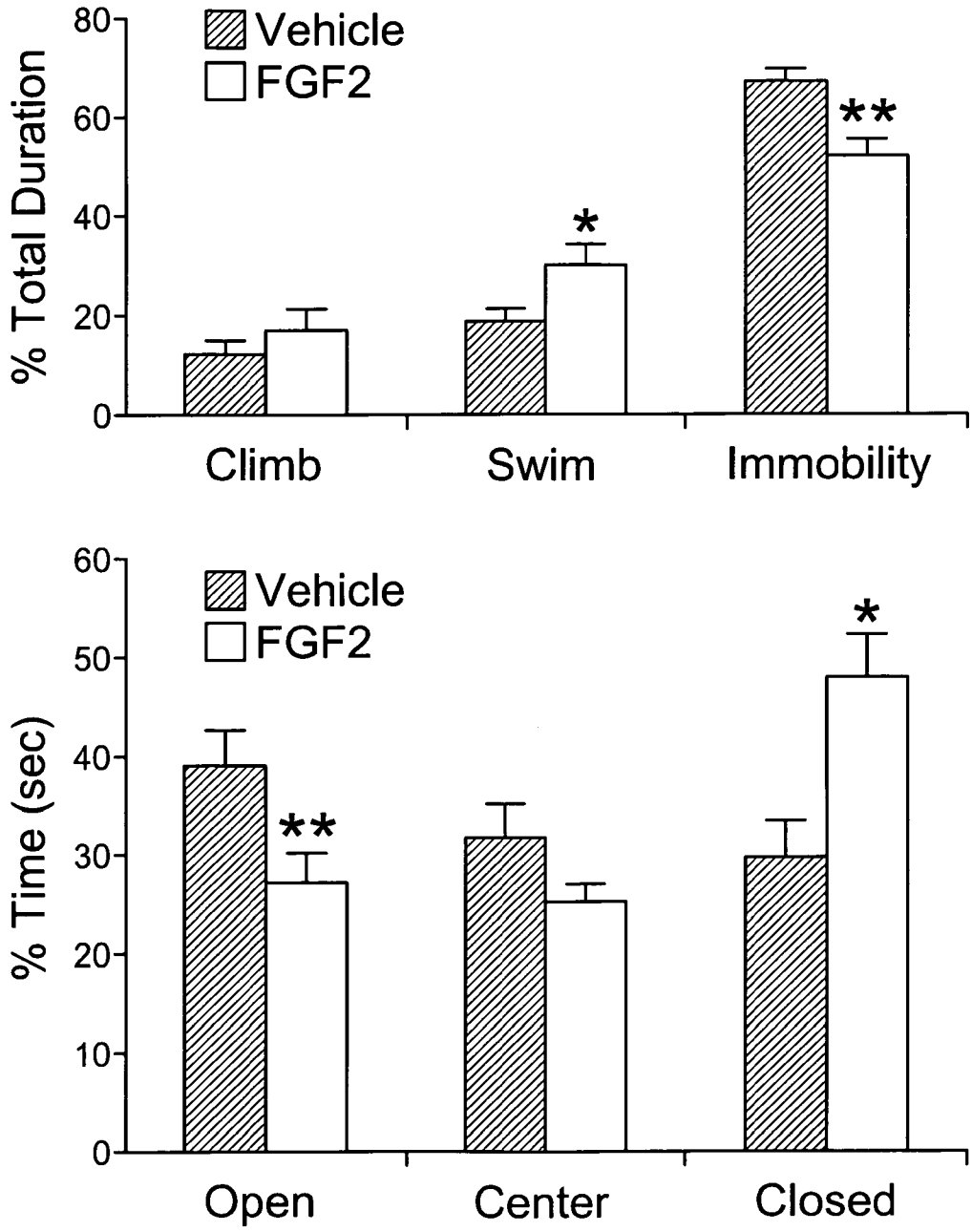


Fig. 3

FST (top) and EPM (bottom)

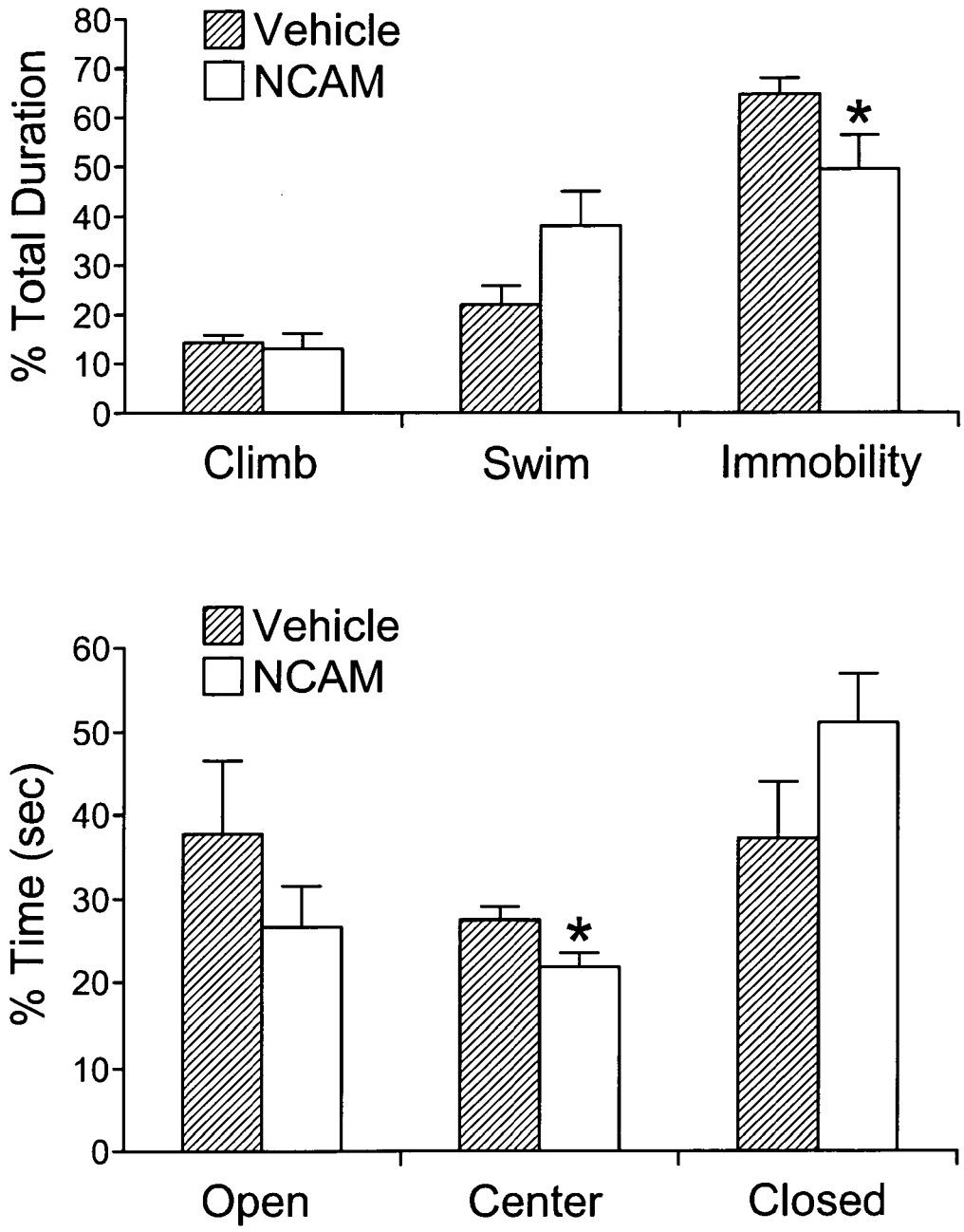


Fig. 4

FST (top) and EPM (bottom)

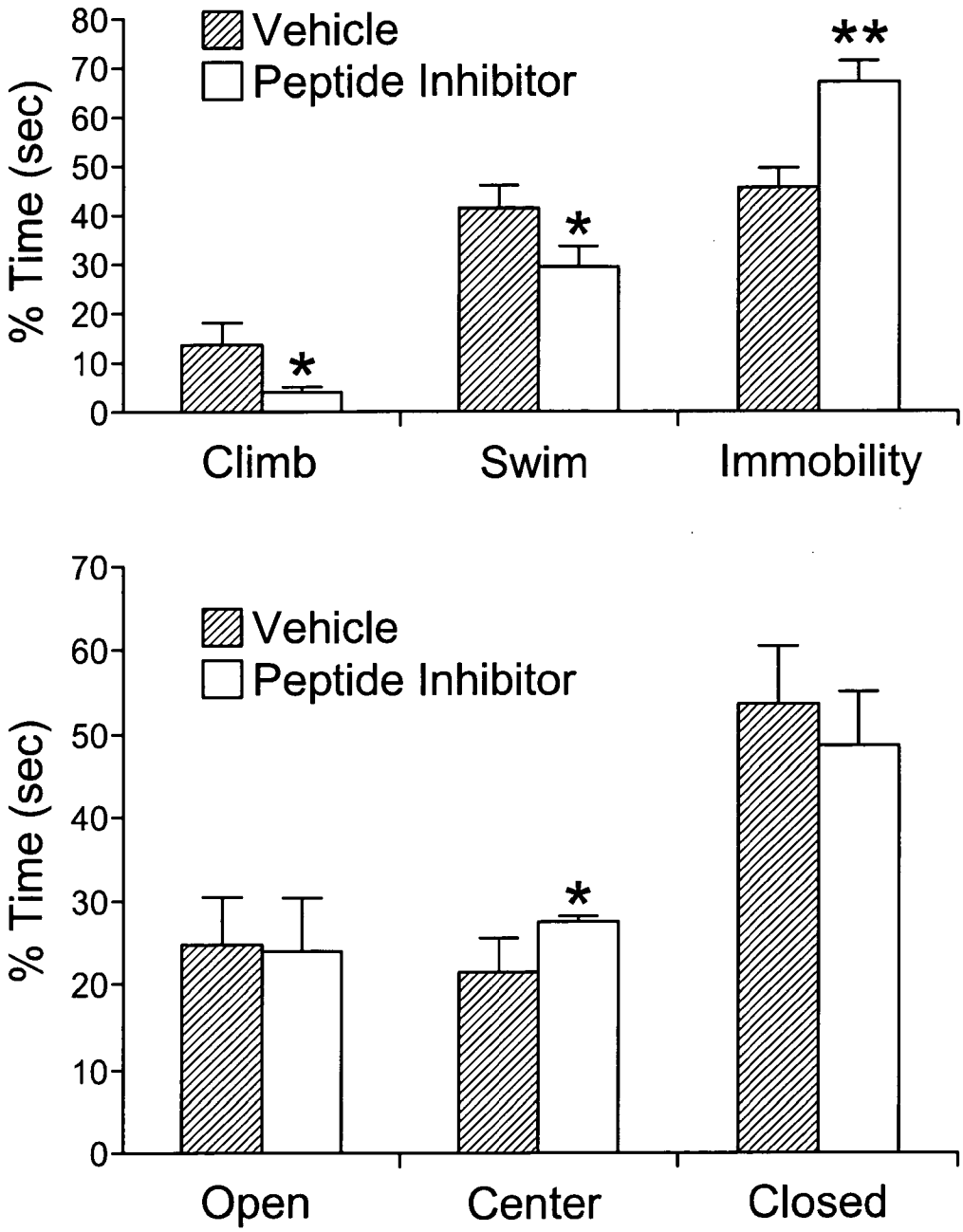


Fig. 5

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. 6

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

Symbol	Name	UniGene ID	Cytoband	MDD Cohort A		MDD Cohort B
				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**	``	-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 signaling 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 ι (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 $p = 0.042$ ($q = 0.36$).

SUBSTITUTE SHEET (RULE 26)

Fig. 8

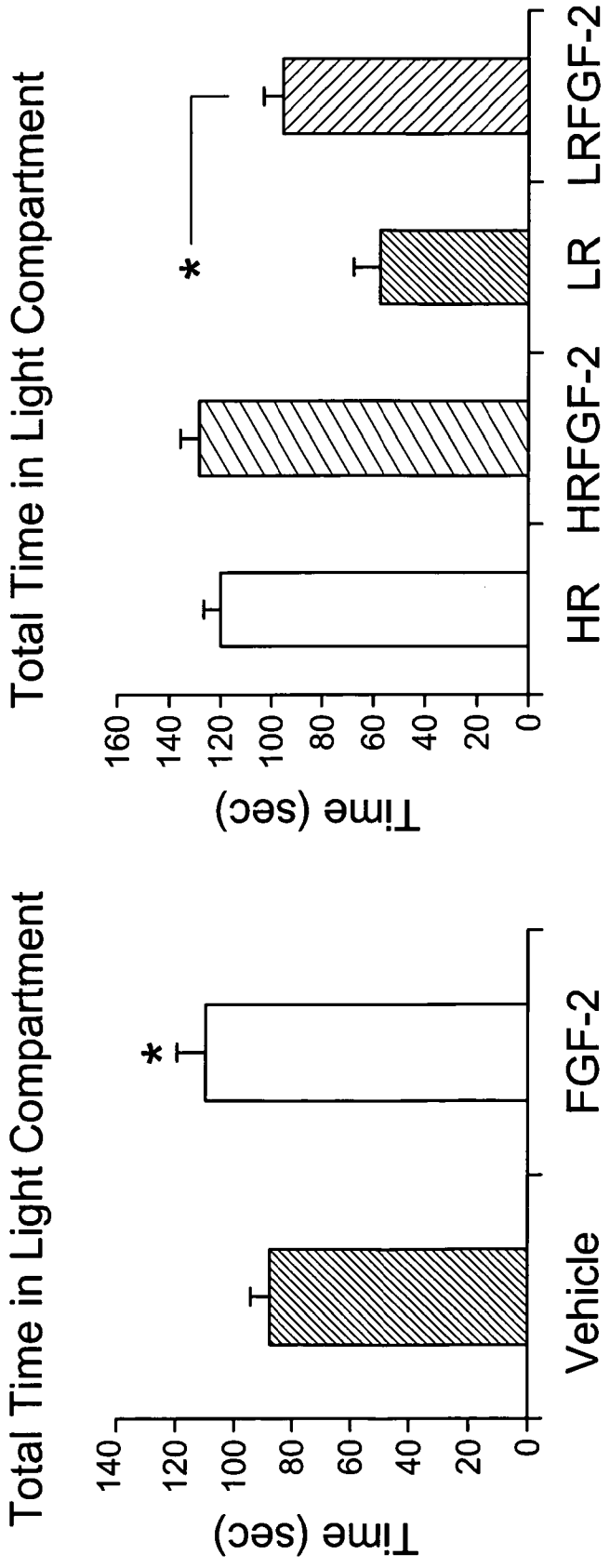
Dysregulation of genes involved in phosphatidylinositol signaling pathways in AnCg from patients with MDD

				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-trisphosphate 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	Phosphoinositide-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


Total Time in Light Compartment
Time (sec)
0
20
40
60
80
100
120
140
160
*
HR
HRFGF-2
LR
LRFGF-2
Total Time in Light Compartment
Time (sec)
0
20
40
60
80
100
120
140
*
Vehicle
FGF-2

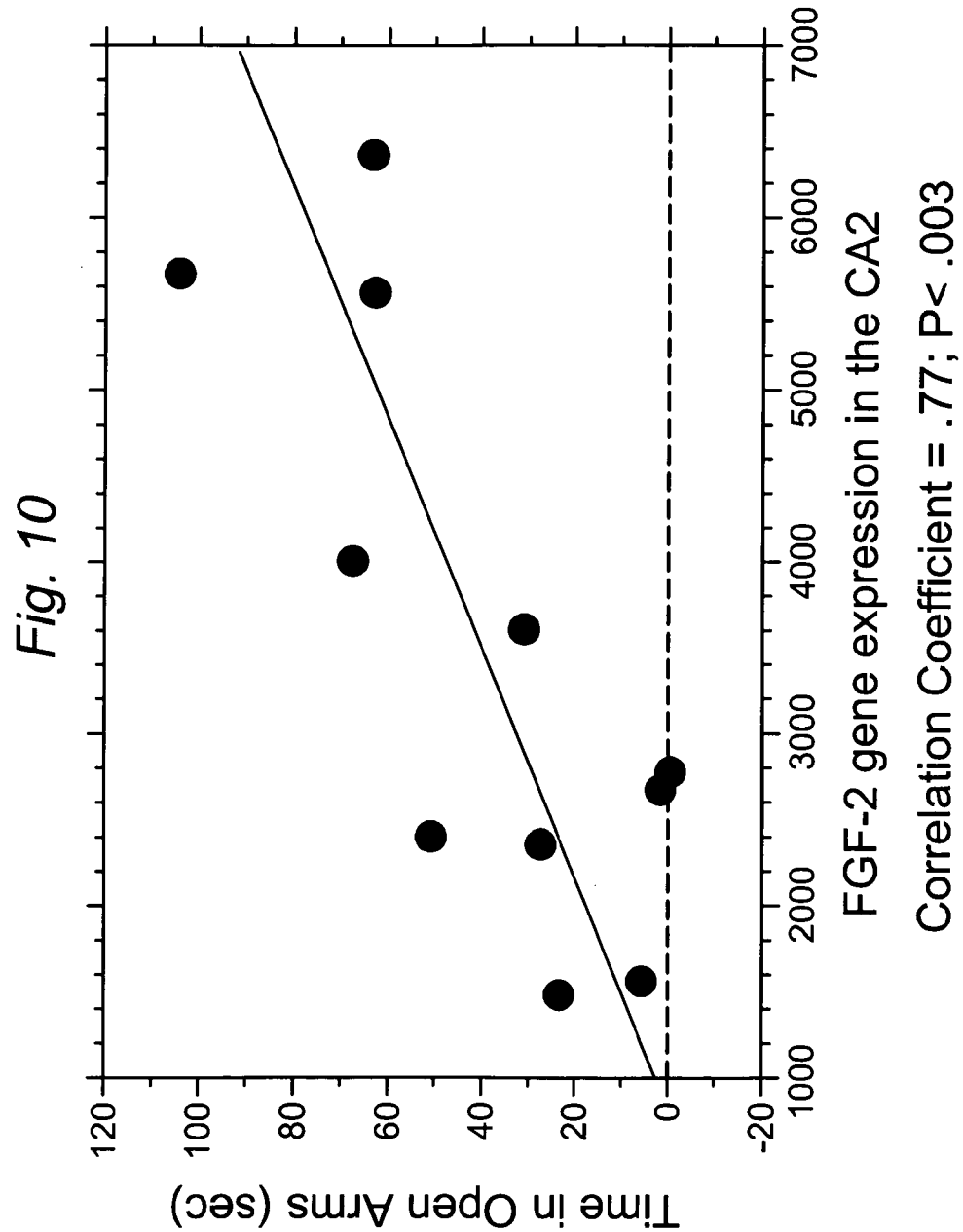

Time in Open Arms (sec)
120
100
80
60
40
20
0
-20
1000
2000
3000
4000
5000
6000
7000
FGF-2 gene expression in the CA2
Correlation Coefficient = .77; P< .003

Fig. 10

Fig. 11 (Sheet 1)

FGF Receptor 2 and 3

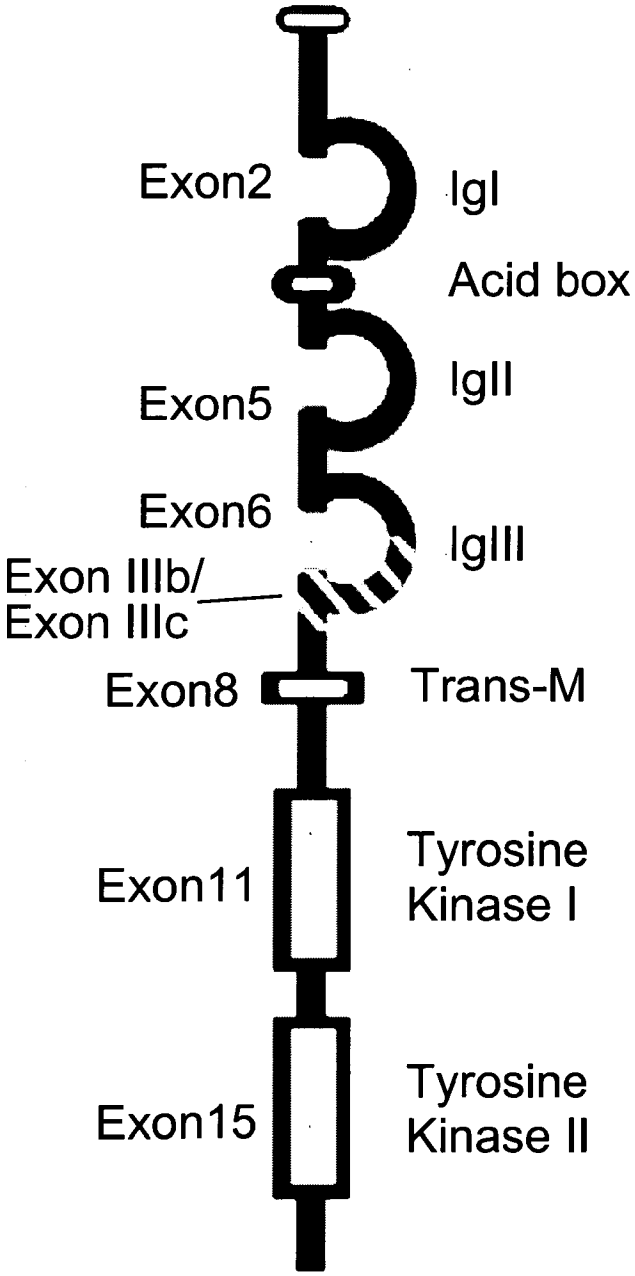
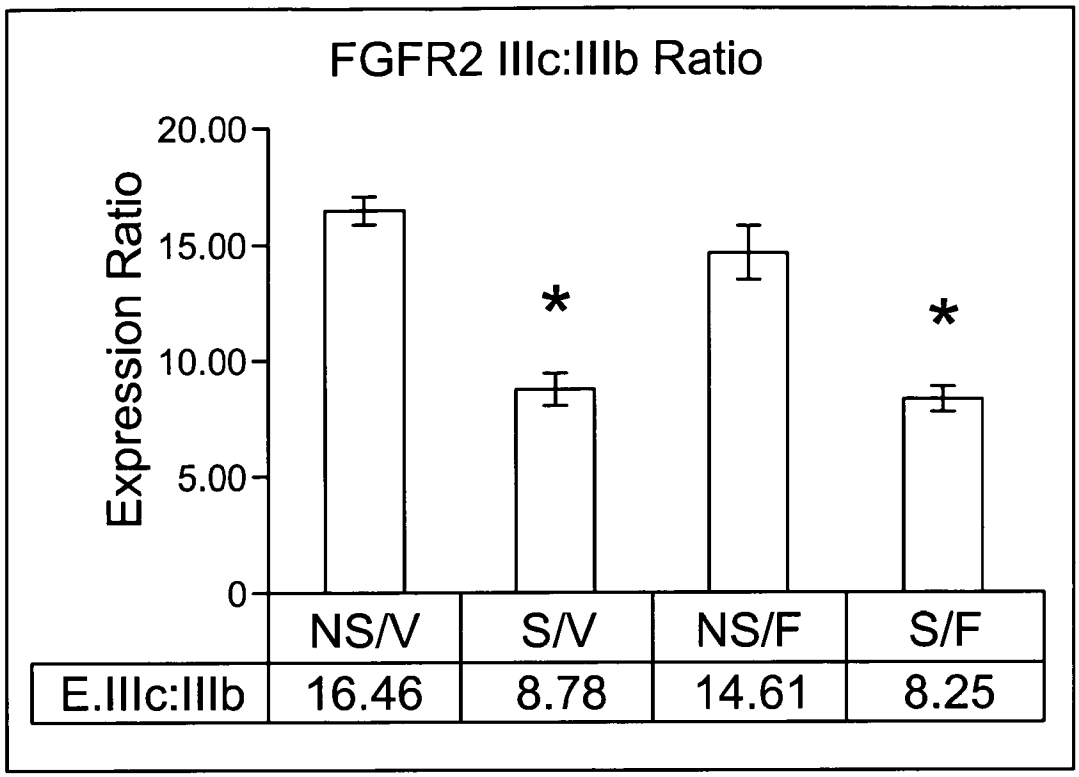


Fig. 11 (Sheet 2)

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
*
*
NS/V
S/V
NS/F
S/F
E.IIIc:IIIb
16.46
8.78
14.61
8.25

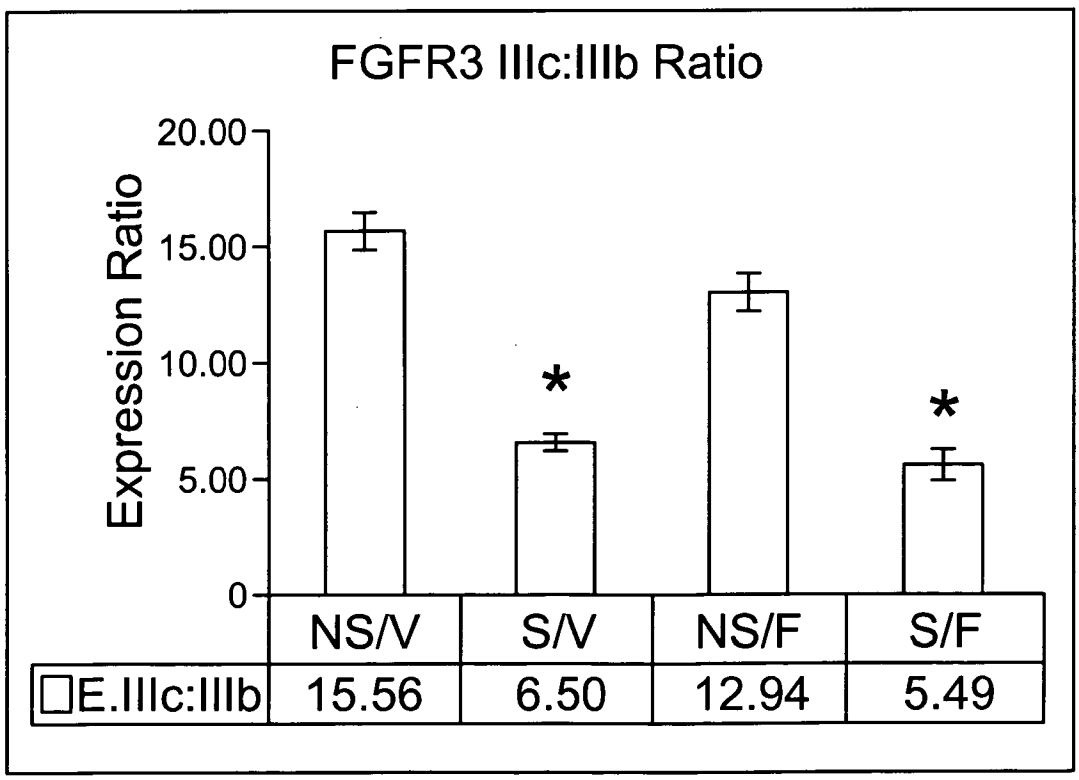

FGFR3 IIIc:IIIb Ratio
Expression Ratio
20.00
15.00
10.00
5.00
0
*
*
NS/V
S/V
NS/F
S/F
E.IIIc:IIIb
15.56
6.50
12.94
5.49

Fig. 13 (Sheet 1)

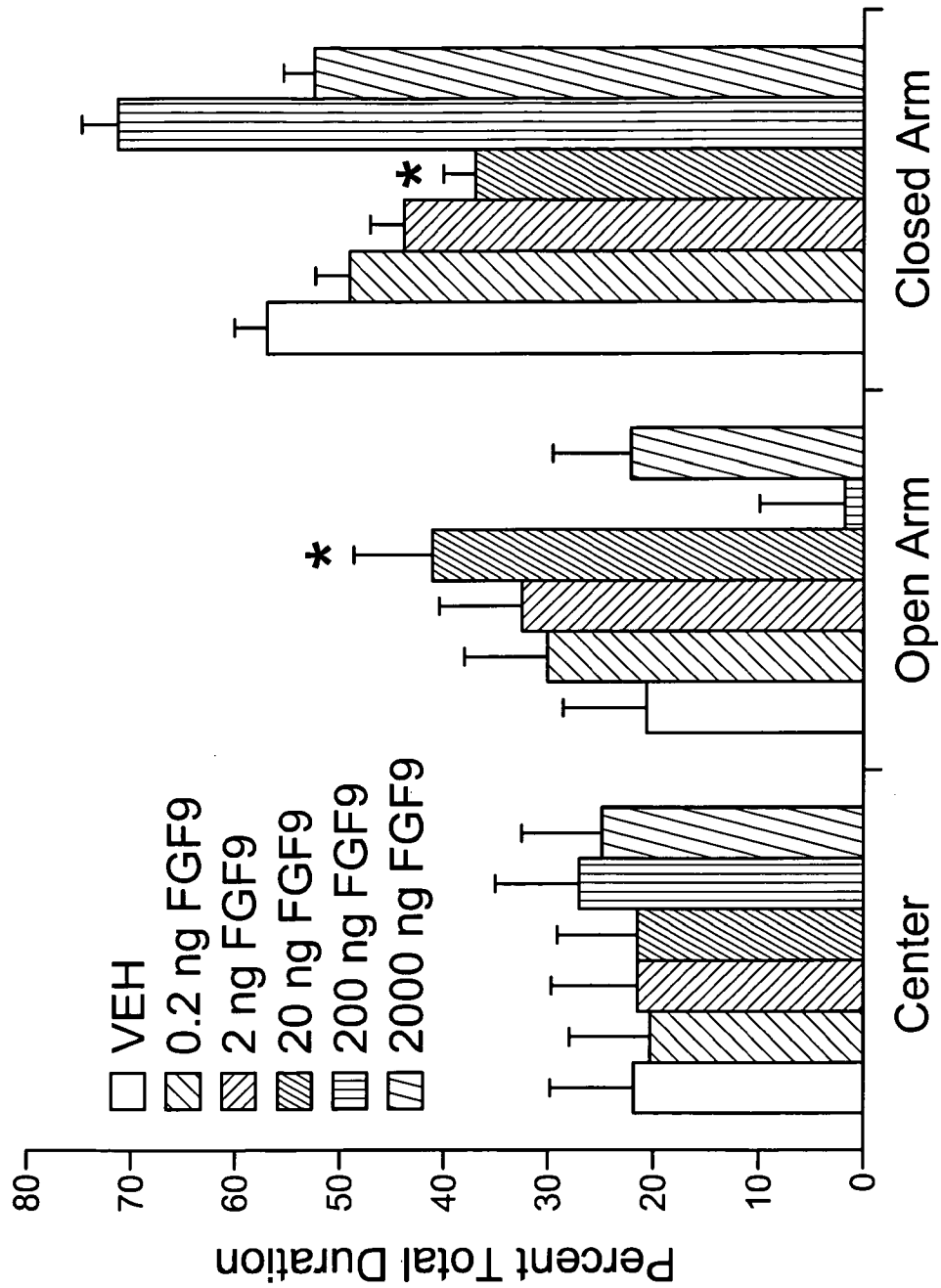


Fig. 13 (Sheet 2)

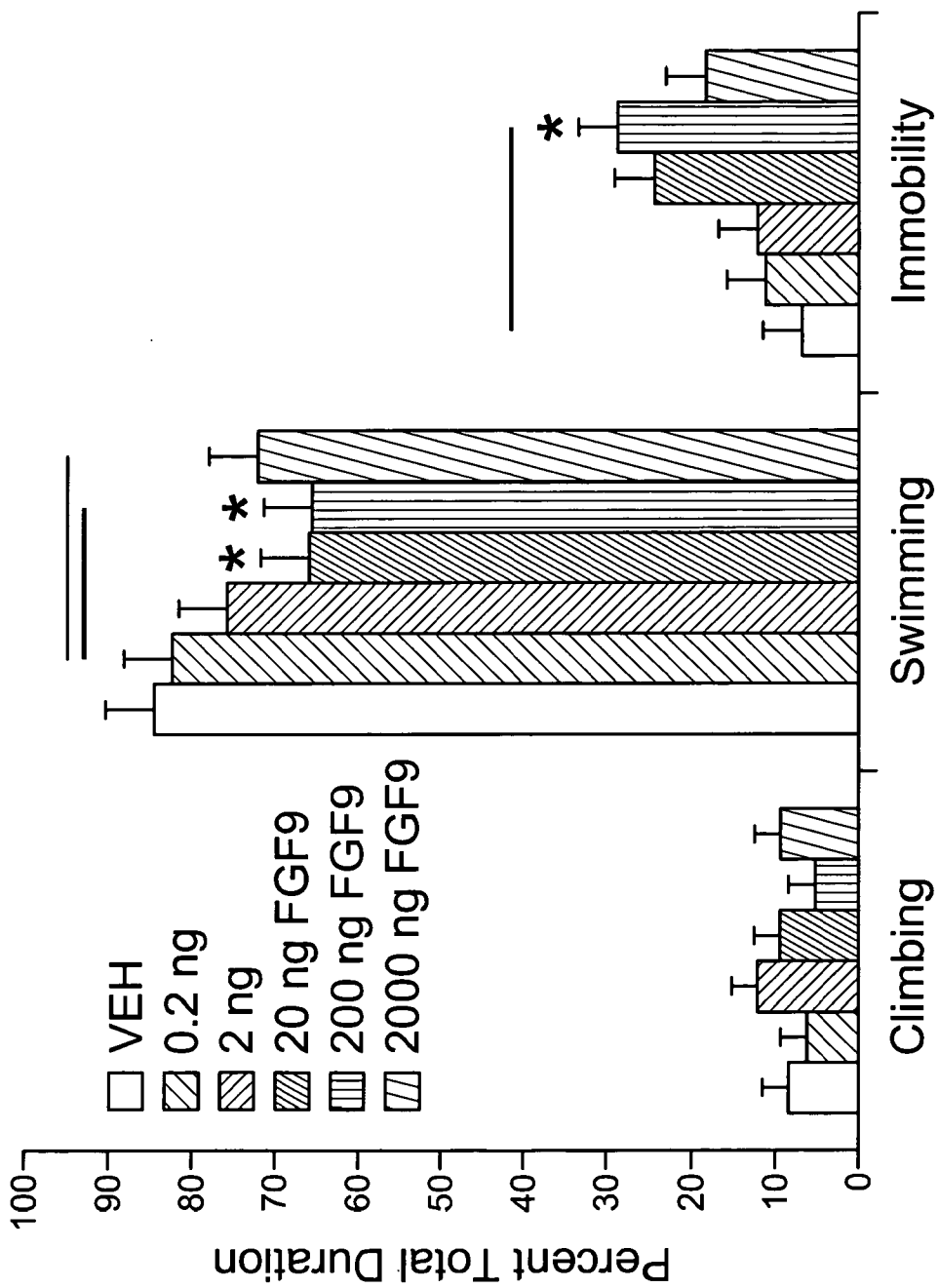
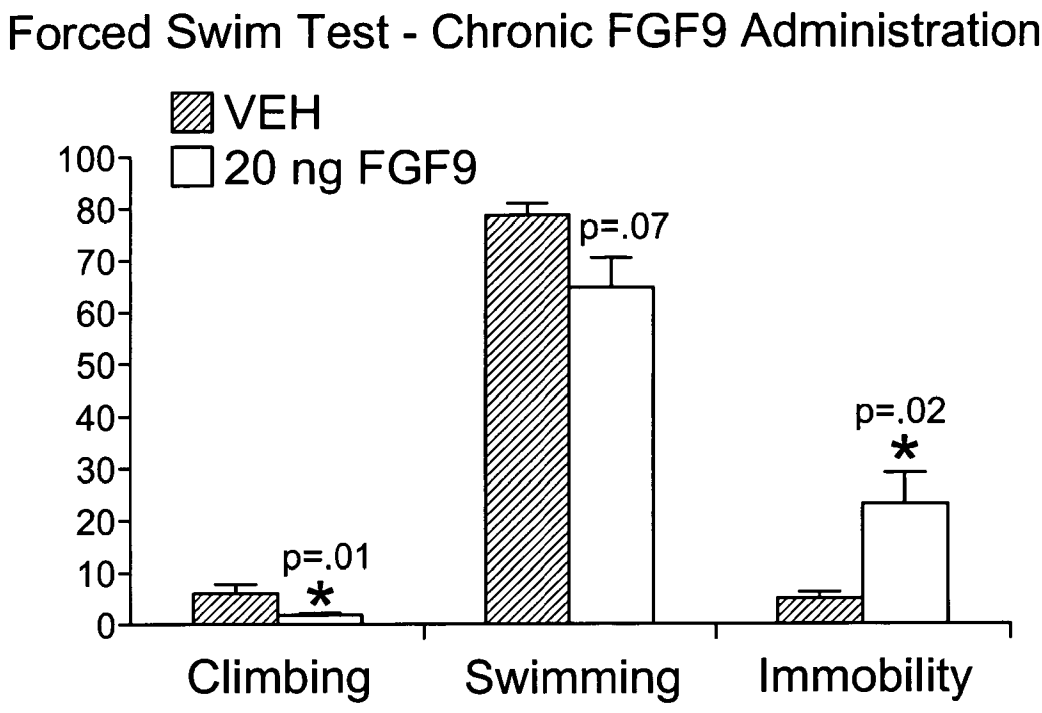
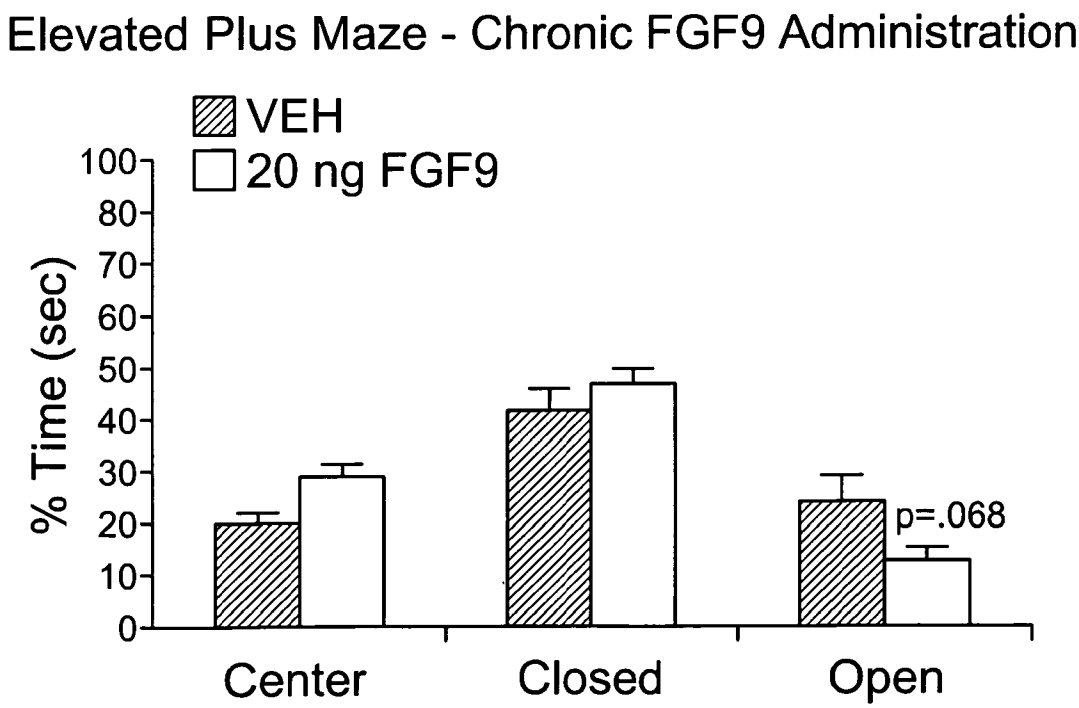


Fig. 14


Forced Swim Test - Chronic FGF9 Administration
VEH
20 ng FGF9
100
80
70
60
50
40
30
20
10
0
p=.01
*
p=.07
p=.02
*
Climbing
Swimming
Immobility


Elevated Plus Maze - Chronic FGF9 Administration
VEH
20 ng FGF9
% Time (sec)
100
80
70
60
50
40
30
20
10
0
p=.068
Center
Closed
Open

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/82279

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00 (2009.1)
USPC - 424/198.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K 39/00 (2009.1)
USPC: 424/198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 530/350, 514/12, 536/23.500, 530/351, 530/399

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest(PGPB,USPT,EPAB,JPAB); Google Scholar
Search Terms: anxiety, depression, FGF-2, FGF2, FGF 2, FGF 9, FGF9, FGF-9, treat, administer, inject

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2002/0169102 A1 (FREY) 14 Nov 2002 (14.11.2002) para [0019], [0020], [0037], [0050], [0076], [0091], [0106]	1, 2, 4-9, 11 ---- 3, 10
Y	Gomez-Pinilla et al. Diazepam induces FGF-2 mRNA in the hippocampus and striatum. Brain Research Bulletin, 2000, Vol. 53, No. 3, pp. 283?289, especially pg 283, col 2, para 1; and the abstract.	10
Y	US 2007/0213401 A2 (DOOLEY, ET AL.) 13 Sept. 2007 (13.09.2007) para [0007]	3
A	Evans, et al., PNAS (101) 43: 15506-15511, 26 Oct 2004 (26.10.2004), pg 15510, col 1, para 2	10

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2009 (23.01.2009)	06 FEB 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)