

Convergent functional genomic studies of omega-3 fatty acids in stress reactivity, bipolar disorder and alcoholism

H Le-Niculescu¹, NJ Case¹, L Hulvershorn¹, SD Patel^{1,4}, D Bowker¹, J Gupta¹, R Bell¹, HJ Edenberg², MT Tsuang³, R Kuczenski³, MA Geyer³, ZA Rodd¹ and AB Niculescu^{1,4}

Omega-3 fatty acids have been proposed as an adjuvant treatment option in psychiatric disorders. Given their other health benefits and their relative lack of toxicity, teratogenicity and side effects, they may be particularly useful in children and in females of child-bearing age, especially during pregnancy and postpartum. A comprehensive mechanistic understanding of their effects is needed. Here we report translational studies demonstrating the phenotypic normalization and gene expression effects of dietary omega-3 fatty acids, specifically docosahexaenoic acid (DHA), in a stress-reactive knockout mouse model of bipolar disorder and co-morbid alcoholism, using a bioinformatic convergent functional genomics approach integrating animal model and human data to prioritize disease-relevant genes. Additionally, to validate at a behavioral level the novel observed effects on decreasing alcohol consumption, we also tested the effects of DHA in an independent animal model, alcohol-preferring (P) rats, a well-established animal model of alcoholism. Our studies uncover sex differences, brain region-specific effects and blood biomarkers that may underpin the effects of DHA. Of note, DHA modulates some of the same genes targeted by current psychotropic medications, as well as increases myelin-related gene expression. Myelin-related gene expression decrease is a common, if nonspecific, denominator of neuropsychiatric disorders. In conclusion, our work supports the potential utility of omega-3 fatty acids, specifically DHA, for a spectrum of psychiatric disorders such as stress disorders, bipolar disorder, alcoholism and beyond.

Translational Psychiatry (2011) 1, e4; doi:10.1038/tp.2011.1; published online 26 April 2011

Introduction

'First do no harm'

—Hippocratic Oath

There is a strong need for better treatments, with less side effects, for stress, mood and alcohol use disorders. Natural compounds may offer a source for such treatments, but have been in general insufficiently studied in preclinical models, and a molecular understanding is lacking. Omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid (DHA)) are essential fatty acids, with DHA being the final metabolic pathway compound. They have been speculated to have had an evolutionary role in the development of the brain in higher organisms,¹ and their relative depletion compared with proinflammatory omega-6 fatty acids in modern Western diets has been invoked as having a role in the pathophysiology of multiple diseases.² Omega-3 fatty acids, particularly DHA, have been described to have mood- and psychosis-modulating properties, in both preclinical models and some clinical trials. For example, deficits in omega-3 fatty acids have been linked to increased depression and aggression in animal models^{3,4} and humans.^{5,6} Of note, deficits in DHA have been reported in erythrocytes⁷ and in the post-mortem

orbitofrontal cortex of patients with bipolar disorder, and were greater in those who had high vs those who had low alcohol abuse.⁸ Omega-3 fatty acids have been reported to be clinically useful in the treatment of both mood^{9–12} and psychotic disorders.^{13–15} To date, there is no clear understanding of how they work in terms of psychotropic effects, or indeed how well they actually work. Unlike most psychiatric drugs, these natural compounds have minimal side effects, and intriguing evidence for favorable health benefits.^{16–18} Particularly for children and female patients of child-bearing age, the potential developmental and teratogenic side effects of mood-stabilizing and antidepressant medications are a major issue. As such, if the action of omega-3 fatty acids in mood disorders and other related disorders could be substantiated by understanding their mechanistic effects and the identification of candidate molecular biomarkers for treatment response, they would become an important consideration as an addition to the therapeutic armamentarium of psychiatrists, pediatricians and primary care doctors.

We have previously identified the circadian clock gene *D-box binding protein (DBP)* as a potential candidate gene for bipolar disorder,¹⁹ as well as for alcoholism²⁰ and schizophrenia,²¹ using a convergent functional genomics

¹Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA; ²Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA; ³Department of Psychiatry, UC San Diego, La Jolla, CA, USA and ⁴Indianapolis VA Medical Center, Indianapolis, IN, USA
Correspondence: Professor AB Niculescu, Department of Psychiatry, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202, USA.
E-mail: anicules@iupui.edu

Keywords: alcoholism; bipolar; DHA; genomics; omega-3; stress

Received 20 October 2010; accepted 24 February 2011

(CFG) approach. In follow-up work, we established mice with a homozygous deletion of DBP (DBP knockout (KO)) as a stress-reactive genetic animal model of bipolar disorder and co-morbid alcoholism.²² We reported that DBP KO mice have lower locomotor activity and blunted responses to stimulants and they gain less weight over time. In response to a chronic stress paradigm, the mice exhibit a diametric switch in these phenotypes. DBP KO mice are also activated by sleep deprivation, similar to bipolar patients, and that activation is prevented by treatment with the mood stabilizer drug valproate. Moreover, these mice show increased alcohol intake following exposure to stress. Microarray studies of brain and blood revealed a pattern of gene expression changes that may explain the observed phenotypes. CFG analysis of the gene expression changes identified a series of novel candidate genes and blood biomarkers for bipolar disorder, alcoholism and stress reactivity.

Based on the above, we decided to test omega-3 fatty acids, specifically DHA, at a phenotypic, gene expression and blood biomarker level, in this animal model (DBP KO mice subjected to a chronic stress paradigm), using a case–case design²³ to increase signal detection and focus on the effects of DHA. We also studied the effects of DHA on modulating alcohol consumption in these mice and in an independent animal model, the alcohol-preferring (P) rats, a well-established model of alcoholism. Of note, there is a high degree of co-morbidity of alcoholism with depression^{24,25} as well as with bipolar disorder.²⁶ The work described has important translational implications for understanding and validating a new treatment approach, which follows the Hippocratic principle of ‘first do no harm’ and may favorably impact multiple co-morbid medical and psychiatric conditions.

Materials and methods

Mouse colony. The generation of transgenic mice carrying DBP-KO has been previously described in detail.²² DBP (+/–) heterozygous (HET) mice were bred to obtain mixed littermate cohorts of DBP (+/+) wild-type (WT), HET and DBP (–/–) KO mice. Mouse pups were weaned at 21 days and housed by gender in groups of two to four in a temperature- and light-controlled colony on reverse cycle (lights on at 2200 h, lights off at 1000 h), with food and water available *ad libitum*. DNA for genotyping was extracted by tail digestion with a Qiagen DNeasy Tissue kit, following the protocol for animal tissue (Qiagen, Valencia, CA, USA). The following three primers were used for genotyping by PCR: Dbp forward: 5'-TTCTTTGGGCTTGCTGTTTCCCTGCAGA-3'/ Dbp reverse: 5'-GCAAAGCTCCTTTCTTTCGCGAGAAGTGC-3' (WT allele) lacZ reverse: 5'-GTGCTGCAAGGCGATTAAGTTGGGTAAC-3' (KO allele) WT or KO mice, 8–12 weeks old, were used for experiments.

Animal housing, diets and treatment. All mice were housed for at least 1 week before each experiment in a room set to an alternating light cycle with 12 h of darkness from 1000 to 2200 h, and 12 h of light from 2200 to 1000 h. At the start of the experiment, male and female DBP (+/+) WT or DBP (–/–) KO mice were placed on one of the two diets:

(1) low DHA custom research diet (TD 00522, Harlan Teklad, Madison, WI, USA), a DHA-depleting low n-3 PUFA test diet adequate in all other nutrients (n-6/n-3 ratio of 85:1 with 6% fat as safflower oil);²⁷ or (2) high DHA custom research diet (TD 07708 low-DHA diet supplemented with 0.69% algal DHA; Martek Bioscience, Columbia, MD, USA).²⁷ The DBP mice were fed the low-DHA diet (0% DHA) or high-DHA diet (0.69% DHA) for 28 days. Mice and food and water were weighed twice a week. Water was refilled once a week.

Mice were subjected to a chronic stress paradigm consisting of isolation (single housing) for 28 days, with an acute stressor (behavioral challenge tests) on day 21. The behavioral challenge tests consisted of sequential administration of the forced swim test (FST), tail flick test and tail suspension test.

At 4 weeks (day 28), the mice were injected with saline to keep handling consistent with previous work²² and their open field locomotor activity was assessed with SMART II video-tracking software (San Diego Instruments, San Diego, CA, USA). After video tracking, brain and blood were harvested as previously described²² for use in microarray studies.

Behavioral challenge tests

Forced Swim Test. FST experiments were performed on day 21 of treatment during the dark cycle. Mice were placed one at a time in a transparent plexiglas cylinder (64 cm height × 38 cm diameter), with water depth of 30 cm and temperature of 23 ± 2 °C. Water was replaced after each mouse tested. Time spent immobile in a 10-min interval was scored live by two independent observers blinded to the genotype and treatment group of the animals.

Tail flick. Immediately following the FST, the mice were dried with paper towels and placed in the Plexiglas chamber of the Tail Flick Analgesia Meter System (San Diego Instruments). The mouse's tail was placed over a window located on the Tail Flick platform where a light beam shines to heat the tail at a reliable, reproducible rate for 20 ± 1 s. This test was performed as an acute stressor, and not as a way to measure the mouse's response to pain, as it is confounded by the preceding test.

Tail suspension. For the third part of the acute stress paradigm, the mouse was suspended by its tail, ~30 cm above the ground for 5 min. This test was performed as an acute stressor, and not as a way to measure the mouse's behavior, as it is confounded by the preceding tests.

Locomotion testing. A SMART II Video Tracker (VT) system (San Diego Instruments) under normal light was used to track the movement of mice. The mice were placed in the lower-right-hand corner of one of four adjacent, 41 × 41 × 34 cm³ enclosures. Mice were not allowed any physical contact with other mice during testing. Each enclosure had nine predefined areas, that is, center area, corner area and wall area. The movements of the mice were recorded for 30 min. The enclosures were cleaned with water after each tracking. Measures of total distance traveled, center entry, center time, fast movement, resting time,

average velocity (V mean) and maximum velocity (V max) were obtained.

Clustering analysis of locomotion pattern using GeneSpring. GeneSpring GX (Agilent Technologies, Palo Alto, CA, USA), the most widely used, commercially available, microarray gene expression analysis software, was adapted for the novel use of analyzing and visualizing phenotypic data. We have inputted the scores on phenotypic items numbers in lieu of the usual use of gene expression intensity numbers. All the subsequent analyses were carried out using the same tools as for gene expression data sets, as per the manufacturer's instructions (www.chem.agilent.com). Unsupervised two-way hierarchical clustering of normalized (Z -scored) behavioral data from locomotor testing was carried out using methodology previously described.^{22,28}

Alcohol consumption experiments in mice. To create an alcohol free-choice drinking paradigm, male DBP (+/+) WT or DBP (-/-) KO mice were placed in individual cages with both a bottle of ~250 ml cold tap water and a bottle of ~250 ml 10% ethanol, the customary concentration used in mouse studies of alcohol consumption, and either a low- or high-DHA diet for 28 days, with an acute stressor (behavior challenge tests described above) on day 21. The amount of ethanol and water consumed was recorded twice a week, at which time the locations of the bottles were switched to prevent positional bias. The bottles were refilled with fresh solution once a week.

Alcohol consumption experiments in alcohol-preferring (P) rats. Experimentally naive, male P rats, 4–6 months of age at the start of the experiment, were used as subjects. They were placed on three diets (1) low DHA custom research diet (TD 00522, Harlan Teklad); (2) high omega-3 custom research diet (TD 07708, 0.69% DHA), similar to the DBP KO mice experiments; and (3) normal rat diet (7001, Harlan Teklad) for a duration of 28 days. Food and water were available *ad libitum* throughout the experiments. Rats were given continuous free-choice access in the home cage to 15% v/v ethanol and water, the customary concentration used in rat studies of alcohol consumption. Ethanol intake was measured daily throughout the experiment.

Behavioral statistical analysis. Behavioral data are expressed as the mean \pm s.e.m. Two-way analysis of variance was used to determine statistically significant differences for factors of gender, genotype and diet, using SPSS statistical software (SPSS, Chicago, IL, USA). We used a one-tailed, two-sample independent t -tests assuming unequal variance to determine significant differences between individual groups. Differences between groups were considered significant at a $P < 0.05$ (Figure 1).

RNA extraction and microarray work. Following the locomotor behavioral testing, mice were sacrificed by cervical dislocation, then they were decapitated and blood was collected. Behavioral testing and tissue harvesting were done at the same time of day in all experiments. The brains of the mice were harvested, stereotactically sliced, and hand

microdissected using Paxinos mouse anatomical atlas coordinates, to isolate anatomical regions of interest—prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HIP).^{21,29} Tissues were flash frozen in liquid nitrogen and stored at -80°C pending RNA extraction. Approximately 0.5–1 ml of blood per mouse was collected into a PAXgene blood RNA collection tubes (BD Diagnostics, Franklin Lakes, NJ, USA). The PAXgene blood vials were stored in -4°C overnight, and then at -80°C until future processing for RNA extraction.

Standard techniques were used to obtain total RNA (22-gauge syringe homogenization in RLT buffer) and to purify the RNA (RNeasy mini kit, Qiagen) from microdissected mouse brain regions. For the whole mouse blood RNA extraction, PAXgene blood RNA extraction kit (PreAnalytiX, a QIAGEN/BD company, BD Diagnostics) was used, followed by GLOBINclear-Mouse/Rat (Ambion/Applied Biosystems, Austin, TX, USA) to remove the globin mRNA. All the methods and procedures were carried out as per the manufacturer's instructions. The quality of the total RNA was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies). The quantity and quality of total RNA was also independently assessed by 260 nm ultraviolet absorption and by 260/280 ratios, respectively (Nanodrop spectrophotometer, Thermo Scientific, Wilmington, DE, USA). Starting material of total RNA labeling reactions was kept consistent within each independent microarray experiment.

Equal amounts of total RNA extracted from the brain tissue samples or blood from three mice per group was pooled for each experimental condition and used for labeling and hybridization to Mouse Genome 430 2.0 arrays (Affymetrix, Santa Clara, CA, USA). The GeneChip Mouse Genome 430 2.0 Array contains over 45 000 probe sets that analyze the expression level of over 39 000 transcripts and variants from over 34 000 well-characterized mouse genes. Standard Affymetrix protocols were used to reverse transcribe the messenger RNA and generate biotinylated cRNA (http://www.affymetrix.com/support/downloads/manuals/expression_s2_manual.pdf). The amount of cRNA used to prepare the hybridization cocktail was kept constant within each experiment. Samples were hybridized at 45°C for 17 h under constant rotation. Arrays were washed and stained using the Affymetrix Fluidics Station 400 and scanned using the Affymetrix Model 3000 Scanner controlled by GCOS software. All sample labeling, hybridization, staining and scanning procedures were carried out as per the manufacturer's recommendations.

Quality control. All arrays were scaled to a target intensity of 1000 using Affymetrix MASv 5.0 array analysis software. Quality control measures including 3'/5' ratios for glyceraldehyde 3-phosphate dehydrogenase and β -actin, scaling factors, background and Q values were used.

Microarray data analysis. Data analysis was performed using Affymetrix Microarray Suite 5.0 software (MAS v5.0). Default settings were used to define transcripts as present (P), marginal (M) or absent (A). A comparison analysis was performed for DBP KO mice on high-DHA diet, using DBP KO mice on low-DHA diet as the baseline. 'Signal',

'Detection', 'Signal Log Ratio', 'Change' and 'Change P -value' were obtained from this analysis. An empirical P -value threshold for change of $P < 0.0025$ was used. Only transcripts that were called Present and that were reproducibly changed in the same direction in two independent experiments were analyzed further.

Gene identification. The identities of transcripts was established using NetAFFX (Affymetrix), and confirmed by cross-checking the target mRNA sequences that had been used for probe design in the Affymetrix Mouse Genome 430 2.0 arrays GeneChip with the GenBank database. Probe sets that did not have a known gene are labeled 'EST' and their accession numbers kept as identifiers.

Convergent Functional Genomics analyses

Databases. We have established in our laboratory (Laboratory of Neurophenomics, IU School of Medicine) manually curated databases of all the human gene expression (postmortem brain, blood), human genetic

(association, linkage) and animal model gene expression studies published to date on psychiatric disorders. These constantly updated large databases have been used in our CFG cross-validation (Figure 2).

Human genetic evidence (linkage, association). To designate convergence for a particular gene, the gene had to map within 10 cM (see ref. 19 for detailed discussion) of a microsatellite marker for which at least one published study showed evidence of genetic linkage or a positive association study for the gene itself was reported in the literature (for bipolar disorder, depression, alcoholism, stress and anxiety). The University of Southampton's sequence-based integrated map of the human genome (The Genetic Epidemiological Group, Human Genetics Division, University of Southampton: http://cedar.genetics.soton.ac.uk/public_html/) was used to obtain cM locations for both genes and markers. The sex-averaged cM value was calculated and used to determine convergence to a particular marker. For markers that were not present in the Southampton database,

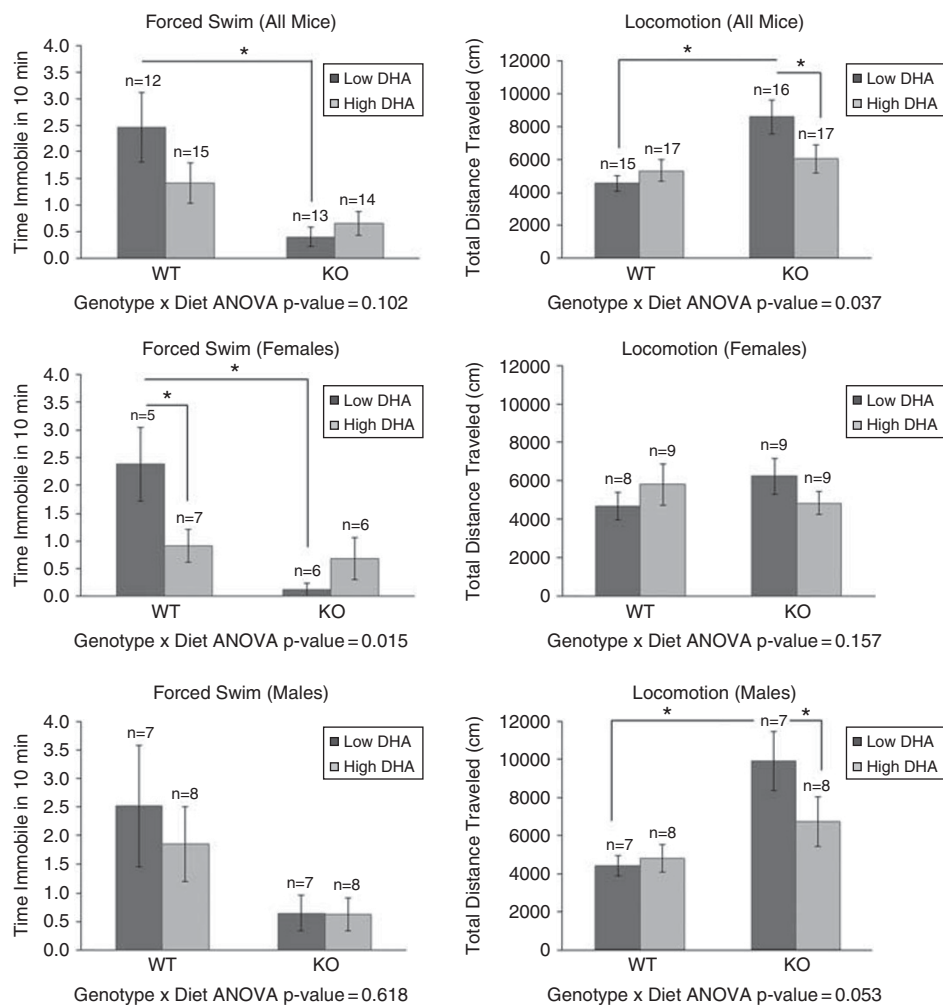


Figure 1 Effects of docosahexaenoic acid (DHA) on stressed mice behavior: DBP (+/+) wild-type (WT) and DBP (-/-) knockout (KO) mice on a diet either high or low in DHA were subjected to a chronic stress paradigm consisting of isolation (single housing) for 28 days, with an acute stressor (behavioral challenge tests, including forced swim test) at day 21. On day 28, video-tracking software was used to measure locomotion (total distance traveled, in centimeters) during a 30-min period in open field. Two-factor analysis of variance (ANOVA) was done for genotype and diet. Additionally, one-tail t -tests with $*P < 0.05$ are depicted.

the Marshfield database (Center for Medical Genetics, Marshfield, WI, USA: <http://research.marshfieldclinic.org/genetics>) was used with the NCBI (National Center for Biotechnology Information) Map Viewer website to evaluate linkage convergence.

Human gene expression evidence (post-mortem brain, blood). Information about our candidate genes was obtained using GeneCards, the Online Mendelian Inheritance of Man database (<http://ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>), as well as database searches using PubMed (<http://ncbi.nlm.nih.gov/PubMed>) and various combinations of keywords (gene name, bipolar, depression, alcoholism, stress, anxiety, brain, blood, lymphocytes). In addition to our own blood biomarker data for mood disorders,³⁰ we also cross-matched with data for human blood biomarkers for hallucinations and delusions,³¹ as such symptoms occur in dissociative states related to stress and anxiety.

Mouse genetic evidence (quantitative trait loci (QTLs), transgenic). To search for mouse genetic evidence—QTLs or transgenic—for our candidate genes, we utilized the MGI_3.54—Mouse Genome Informatics (Jackson Laboratory, Bar Harbor, ME, USA) and used the search menu for mouse phenotypes and mouse models of human disease/abnormal behaviors, using the following subcategories: abnormal emotion/affect behavior and abnormal sleep pattern/circadian rhythm, addiction and drug abuse. To designate convergence for a particular gene, the gene had to map within 10 cM of a QTL marker for the abnormal behavior, or a transgenic mouse of the gene itself displayed that behavior.

Animal model gene expression evidence (brain, blood). Manually curated databases, developed in our lab, of published gene expression studies in animal models of bipolar disorder, depression, alcoholism, stress and anxiety were used for cross-matching with our list of genes changed in expression by DHA in the DBP KO mice (data from studies published by our own group received 1 point, whereas studies published by other groups received 0.5 points).

Convergent Functional Genomics (CFG) scoring. Only genes reproducibly changed in expression in the same mouse tissue (PFC, AMY, HIP, blood), in the same direction, in two independent experiments, were analyzed further. The six external cross-validating lines of evidence (three animal model, three human) were: animal model genetic data, animal model brain gene expression data, animal model blood gene expression data, human genetic data, human brain gene expression data and human blood gene expression data (see Figure 2). These lines of evidence received a maximum of 1 point each (for animal model genetic data, 0.5 points if it was QTL, 1 point if it was transgenic; for human genetic data, 0.5 points if it was linkage, 1 point if it was association). Thus, the maximum possible CFG score for each gene was 6. It has not escaped our attention that other ways of weighing the scores of line of evidence may give slightly different results in terms of prioritization, if not in terms of the list of genes *per se*.

Nevertheless, we feel this simple scoring system provides a good separation and prioritization of genes and blood biomarkers that may be disease relevant, which is our stated focus.

Pathway analyses. Ingenuity 8.0 (Ingenuity Systems, Redwood City, CA, USA) was employed to analyze the molecular networks, biological functions and canonical pathways of the DHA-modulated genes, as well as identify which genes modulated by DHA are also the target of existing drugs.

Results

Effects of DHA on mood-related behavioral measures in DBP KO mice

Activity levels. DBP (+/+) WT and DBP (-/-) KO mice on a diet either low or high in DHA were subjected to a chronic stress paradigm consisting of isolation (single housing) for 28 days, with an acute stressor (behavioral challenge tests, including FST) at day 21. On day 28, we measured locomotion in open field. Two-factor analysis of variance was carried out (genotype \times diet) for FST and Open Field Locomotion.

The FST is a standard test used to measure mood state and response to antidepressant medications in rodents. In female mice (Figure 1), we observed a significant decrease in immobility in the depressed-like WT mice, and an increase in immobility in the manic-like KO mice, on high-DHA diet compared with low-DHA diet. In other words, DHA supplementation seemed to normalize mood state, acting as a mood-stabilizing agent. A slight trend toward reducing immobility in WT male mice was also observed.

Open Field Locomotion is a test that is used as a surrogate for mood state, by extrapolation from human behaviors, with higher locomotion corresponding to higher mood, and lower locomotion to lower mood. In male mice (Figure 1), we observed a significant decrease in locomotion in the manic-like KO mice, and a trend to increased locomotion in the depressed-like WT mice, on high-DHA diet compared with low-DHA diet. Again, DHA supplementation seemed to normalize mood state. Similar trends that did not reach significance were observed in female mice.

Two independent behavioral measures related to mood were normalized by DHA treatment, with interesting gender differences observed. The FST was more significantly changed in female mice, and the open field locomotion in male mice. Similar gender-related differences in behavior have also been reported in other animal models of mood disorders,³² and may be reflective of human gender differences in mood phenotypes.^{33,34}

PhenoChipping. An unsupervised two-way hierarchical clustering of the mouse open field locomotor behavioral data measures (phenes) using GeneSpring was carried out²² (Supplementary Figure S1). Male stressed (ST) DBP KO mice on the high-DHA diet and male ST DBP KO mice on the low-DHA diet clustered into two distinct groups. Similar to our previous results for male ST DBP KO vs non-ST DBP KO

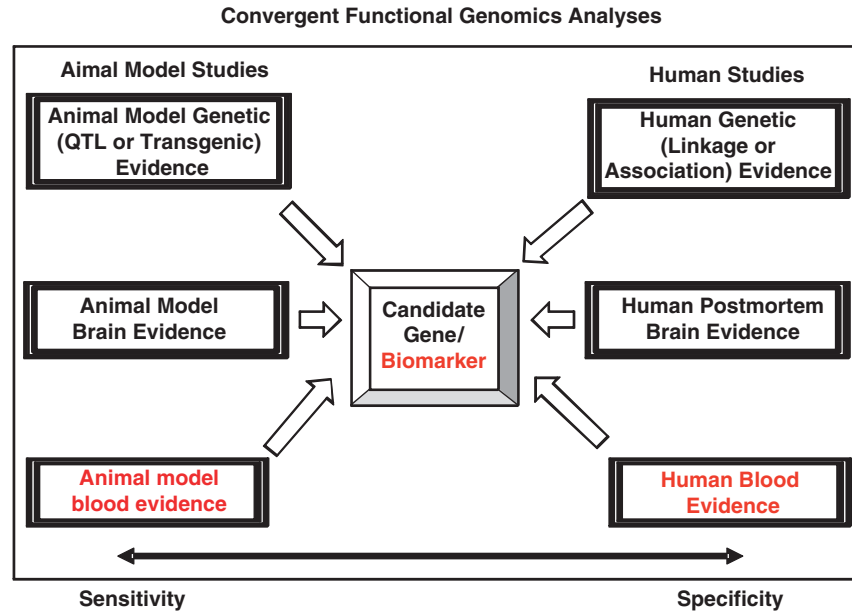


Figure 2 Convergent functional genomics (CFG). Bayesian integration of multiple animal model and human lines of evidence to prioritize disease-relevant genes.

male mice,²² Resting Time was the phenotype most different between male ST DBP KO mice on high- vs low-DHA diet, being increased in the high-DHA diet group. Center Time (time spent in the center quadrant of the open field), was decreased in mice on the high- vs low-DHA diet. A decrease in Center Time may correlate with a decrease in risk-taking behavior or increased anxiety, as mice generally avoid the potentially dangerous, center area of an open field. Female mice did not separate into two distinct clusters.

Food intake. Food is a hedonic stimulus in mice, and the high-DHA diet may be more appetitive than the low-DHA diet because of higher fat content. Total food intake displayed a minimal trend toward increase in high-DHA vs low-DHA diet, irrespective of genotype. The weight changes were in a similar direction, with the notable exception of female DBP WT mice where there was less increase in weight despite increased food intake (Supplementary Figure S2).

Gene expression effects of omega-3 fatty acids in DBP KO mice

Top genes. At the top of our list for disease-relevant genes modulated by DHA in female mice brain (Tables 1 and 2 and Figure 3) are genes such as *GSK3B* (in PFC), *DRD2* and *PPP1R1B/DARPP-32* (in the AMY) and *GRIA2* (in HIP). *GSK3B* (*glycogen synthase kinase 3β*) has consistent signals in genome-wide association studies of bipolar disorder.³⁵ *GSK3B* expression is decreased in mouse PFC by DHA, whereas it is increased in post-mortem human brain in depression.³⁶ Of note, one of the gold standard mood-stabilizing medications for bipolar disorder, lithium, is a *GSK3B* inhibitor.³⁷ *DRD2* (*dopamine receptor 2*) is a main target for numerous antipsychotic medications (Table 5), and *PPP1R1B/DARPP-32* (*protein phosphatase 1, regulatory (inhibitor) subunit 1B/dopamine- and cAMP-regulated*

phosphoprotein, 32 kDa) is at the nexus of signaling pathways by antidepressants and other psychotropic drugs.³⁸ *GRIA2* (*glutamate receptor, ionotropic, AMPA2*) is associated with bipolar disorder,³⁹ and has been reported to be increased in expression in human post-mortem brain from bipolars⁴⁰ and from suicides,⁴¹ whereas DHA decreases the expression in mouse HIP.

At the top of our list for disease-relevant genes modulated by DHA in male mice brain (Tables 1 and 2 and Figure 3) are genes such as *FOS*, *GABRA1*, *MBP* (in HIP) and *PTGDS* (in HIP and PFC). *FOS* (*FBJ osteosarcoma oncogene*) is an immediate response gene involved in response to stress and inflammation. *FOS* is decreased in the mouse PFC by DHA, an effect in opposite direction to the increase seen in post-mortem brains of bipolar subjects,⁴² and in blood cells of subjects with stress disorders.^{43,44} *GABRA1* (*γ-aminobutyric acid (GABA) A receptor, subunit α1*) is associated with bipolar disorder.^{45,46} It is decreased in expression in brains from animal models of alcoholism and stress, whereas DHA increases its expression in DBP mouse HIP. *PTGDS* (*prostaglandin D2 synthase; brain*) is associated with anxiety,⁴⁷ and is decreased in expression in human post-mortem brain from bipolars⁴⁸ as well as in animal models of anxiety⁴⁹ and stress,⁵⁰ whereas DHA increases its expression in the PFC and HIP of DBP KO mice.

Last but not least, *MBP* (*myelin basic protein*) is associated with bipolar disorder, and is decreased in expression in human post-mortem brain from bipolars⁵¹ and from suicides,⁴¹ whereas DHA increases its expression in mouse HIP. Interestingly, a whole series of other myelin-related genes were increased in expression by DHA in DBP male mice (*CNP*, *MOBP*, *PLP1*, *MOG*) and female mice (*MAL*, *PLP1*). Myelin-related gene expression decrease is a common, if nonspecific, denominator of neuropsychiatric disorders,^{51,52} and is modeled by the non-DHA-treated DBP KO mice.²² To our knowledge, DHA is the only compound to date to

Table 1 Top gene expression changes in the brain in female DBP KO ST mice on high-DHA vs low-DHA diet

Gene symbol (name)	PFC change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
GSK3B (glycogen synthase kinase 3β)	D	(I) Alcohol ⁷²		(Transgenic) Behavioral despair	(I) MDD ³⁶	(I) BP ⁷³	3q13.33 (association) MDD ⁷⁴ BP ^{75,76}	5.0
ARHGEF9 (CDC42 guanine nucleotide exchange factor (GEF) 9)	D			(Transgenic) Decreased exploration in new environment	(I) Suicide-MDD ⁴¹	(I) Hallucinations ³¹	Xq11.2 (association) Anxiety ⁷⁷	4.0
GMFB (glia maturation factor, β)	I	(D) DBP-ST PFC ²²	(D) DBP-NST Blood ²²	(QTL) Addiction/drug abuse Abnormal emotion/affect behavior		(I) BP ⁷³	14q22.2 (linkage) Anxiety ⁷⁸	4.0
NFIA (nuclear factor I/A)	D	(I) DBP-NST AMY ²²		(QTL) Abnormal sleep pattern/circadian rhythm Abnormal emotion/affect behavior	(I) MDD ⁷⁹	(I) Alcohol ⁸⁰	1p31.3 (linkage) BP ^{81,82}	4.0
KCNMA1 (potassium large conductance calcium-activated channel, subfamily M, α member 1)	D	(D) DBP-ST PFC ²²		(QTL) Abnormal emotion/affect behavior	(I) MDD ⁷⁹		10q22.3 (association) Alcohol ⁸³	3.5
MGEA5 (meningioma expressed antigen 5)	I	(D) Alcohol ⁸⁴		(QTL) Abnormal circadian rhythm	(D) Alcohol ⁸⁵	(D) Delusion ³¹	10q24.32 (linkage) BP ⁸⁶	3.5
RORB (RAR-related orphan receptor β)	D	(D) DBP-ST PFC; (I) DBP-ST AMY ²²		(Transgenic) Decreased aggression			9q21.13 (association) BP ⁵³ 5q33.3 (linkage) BP ^{86,88,89} PD ⁹⁰	3.0
PTTG1 (pituitary tumor-transforming gene 1)	D	(D) DBP-NST AMY ²²				(I) PPD ⁸⁷		2.5
Gene symbol (name)	AMY change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
DRD2 (dopamine receptor 2)	I	(D) BP ⁹¹ (D) DBP-NST AMY ²²		(Transgenic) Increased drinking behavior; decreased anxiety-related response	(D) MDD ⁹² (D) Alcohol ⁹³	(D) Delusions ³¹	11q23.2 (association) Alcohol ⁹⁴⁻⁹⁸ Anxiety/social phobia ⁹⁹ BP ¹⁰⁰ MDD ⁷⁴ PD ¹⁰¹	5.0
HSPA1B (heat shock protein 1B)	D	(I) Depression ¹⁰² (I) DBP-NST AMY ²²	BP ³⁰	(QTL) Abnormal eating/drinking behavior; abnormal circadian rhythm	(I) Alcohol ¹⁰³	(I) Chronic stress ¹⁰⁴	6p21.33 (linkage) Juvenile BP ¹⁰⁵ Neuroticism ¹⁰⁶	5.0
PPP1R1B (protein phosphatase 1, regulatory (inhibitor) subunit 1B)	I	(D) DBP-NST AMY (I) DBP-ST AMY ²² (I) BP PFC ²⁹		(Transgenic) Abnormal alcohol consumption	(D) BP ¹⁰⁷	(D) BP ¹⁰⁸	17q12 (association) Alcohol ¹⁰⁹	5.0
NOS1 (nitric oxide synthase 1, neuronal)	D	(D) BP ⁹¹ (D) DBP-NST AMY ²²		(QTL) Addiction/drug abuse, Abnormal emotion/affect behavior	(I) BP ¹¹⁰	(D) Stress ¹¹¹	12q24.22 (association) BP ¹¹²	4.5
RGS4 (regulator of G-protein signalling 4)	I	(D) BP ¹¹³		(Transgenic) Abnormal response to addictive substance	(I) Alcohol ¹¹⁴		1q23.3 (association) BP ^{112,115}	4.0

Table 1 (Continued)

Gene symbol (name)	AMY change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>MAL</i> (myelin and lymphocyte protein, T-cell differentiation protein)	I	(D) DBP-NST PFC; (D) DBP-ST PFC ²² Alcohol PFC ¹¹⁶		(QTL) Addiction/drug abuse	(D) MDD ¹¹⁷ (D) Alcohol ¹¹⁴ (D) Suicide-MDD ⁴¹	(D) BP ¹¹⁸	2q11.1 (linkage) MDD-suicide attempts ¹¹⁹ BP ^{120,121}	4.0
<i>ADORA2A</i> (adenosine A2a receptor)	I	BP NAC ²⁹ (D) DBP-ST PFC ²² Alcohol NAC ¹¹⁶		(Transgenic) Decreased exploration in new environment. Abnormal response to addictive substance		(I) Chronic stress ¹⁰⁴	22q11.23 (association) Anxiety ¹²² PD ¹²³⁻¹²⁵	4.0
<i>GALC</i> (galactosylceramidase)	I	(I) DBP-NST AMY ²²		(QTL) Addiction/drug abuse	(D) MDD ¹²⁶ (D) BP ⁵¹	(D) Chronic stress ¹⁰⁴	14q31.3 (linkage) BP ¹²⁷	4.0
<i>PRDX2</i> (peroxiredoxin 2)	I	(D) DBP-ST PFC ²² Alcohol PFC ¹¹⁶	BP ³⁰	(QTL) Abnormal emotion/affect behavior	(D) BP ¹³⁰ (I) Response to lithium treatment ¹³¹		Simple phobia ¹²⁹ 19p13.2 (linkage) BP ¹³²	4.0
<i>TAC1</i> (tachykinin 1)	I	(I) DBP-ST AMY ²²		Abnormal circadian rhythm (Transgenic) Decreased anxiety-related response, increased coping response	(I) MDD ⁷⁹		MDD ¹¹⁹ 7q21.3 (linkage) BP ¹³³	3.5
<i>NR4A3</i> (nuclear receptor subfamily 4, group A, member 3)	D	(I) Alcohol ⁸⁴ (D) MDD- ¹³⁵ Fluoxetine		(QTL) Addiction/drug abuse	(I) Alcohol ¹³⁶	(I) PTSD ⁴³	Alcohol ¹³⁴ 9q22.33 (linkage) Alcohol ¹³⁷	3.5
<i>ESR1</i> (estrogen receptor 1 α)	D	(I) Stress ⁵⁰		(Transgenic) Increased aggression	(I) Alcohol ⁸⁵ (I) MDD ¹³⁹		Anxiety, PD ¹³⁸ 6q25.1 (association) Alcohol ¹⁴⁰	3.5
<i>GABRD</i> (γ -aminobutyric acid (GABA) A receptor, subunit δ)	I	(D) MDD- Fluoxetine ¹³⁵ (I) Anxiety ¹⁴² (D) Alcohol ⁷² Alcohol CP, HIP ¹¹⁶ (D) Depression ¹⁰² (D) DBP-ST PFC ²²		(Transgenic) Decreased anxiety-related response	(I) Suicide-MDD ^{41,143}		18q12.2 (linkage) BP ¹⁴⁴	3.5
<i>CRIM1</i> (cysteine rich transmembrane BMP regulator 1)	D	(D) Anxiety ¹⁴⁶ (D) BP ⁹¹ (D) Stress ⁵⁰ (I) DBP-ST AMY. (D) DBP-ST PFC ²² BP PFC ²⁹		(QTL) Addiction/drug abuse	(D) Suicide-MDD ⁴¹		2p22.3 (association) PD ¹⁴⁵	3.5
<i>PENK</i> (preproenkephalin)	I	(D) Anxiety ¹⁴⁶ (D) BP ⁹¹ (D) Stress ⁵⁰ (I) DBP-ST AMY. (D) DBP-ST PFC ²² BP PFC ²⁹		(Transgenic) Abnormal emotion/affect behavior, addiction/drug abuse	(D) MDD ¹¹⁷ (D) Suicide-MDD ⁴¹		8q12.1 (linkage) BP ¹⁴⁷	3.5
<i>ALDH1A</i> (aldehyde dehydrogenase family 1, subfamily A1)	I	(D) Anxiety/ Depression ¹⁴⁸ (I) DBP-ST AMY ²² Alcohol CP ¹¹⁶	BP ³⁰	(QTL) Abnormal circadian rhythm	(I) Suicide-MDD ⁴¹		9q21.13 (association) Alcohol ¹⁴⁹	3.5
<i>CADPS2</i> (Ca ²⁺ -dependent activator protein for secretion 2)	D	(I) DBP-ST AMY ²² Alcohol CP ¹¹⁶		Decreased exploration in new environment, abnormal circadian rhythm, abnormal sleep pattern			7q31.32 (linkage) BP ¹⁴⁷	3.5
<i>GFRA2</i> (glial cell line derived neurotrophic factor family receptor α , 2)	D	Alcohol NAC ¹¹⁶		(Transgenic) Abnormal food intake, abnormal water consumption	(I) Suicide-MDD ⁴¹		8p21.3 (linkage) MDD/suicide attempts ¹¹⁹	3.5

Table 1 (Continued)

Gene symbol (name)	AMY change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>HPCAL1</i> (hippocalcin-like 1)	D	(D) DBP-ST AMY ²²		(QTL) Abnormal circadian rhythm; addiction/drug abuse	(I) Suicide-MDD ^{41,150}		2p25.1 (association) MDD ¹⁵¹	3.5
Gene symbol (name)	HIP change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>CTNWB1</i> (catenin associated protein), β 1)	D	(I) Anxiety ¹⁵²	BP ³⁰	(Transgenic) Abnormal suckling behavior	(D) Suicide-MDD ⁴¹	(I) Stress ¹¹¹	3p22.1 (linkage) Anxiety/PD ¹³⁸ BP ¹⁵³	5.0
<i>GRIA2</i> (glutamate receptor, ionotropic, AMPA2 α 2)	D	(I) Alcohol ¹⁵⁴ (I) BP ⁹¹ (I) Stress ⁵⁰ (I) Alcohol ⁷² (I) Alcohol ¹¹⁶	BP ³⁰	(Transgenic) Abnormal anxiety-related response	(I) BP ⁴⁰ (I) Suicide-MDD ⁴¹		4q32.1 (association) BP ³⁹	5.0
<i>GJA1</i> (gap junction protein, α 1)	D	(I) Alcohol ¹¹⁶	BP ³⁰	(Transgenic) Abnormal suckling behavior	(I) Alcohol ¹⁰³		6q22.31 (linkage) BP ^{155,156}	4.5
<i>GLUL</i> (glutamate-ammonia ligase glutamine synthetase)	D	(I) Stress ¹⁵⁸ BP PFC, VT ²⁹ Alcohol AMY, NAC ¹¹⁶	BP ³⁰	(QTL) Abnormal emotion/affect behavior, Addiction/drug abuse	(D) MDD ^{159,160} (D) Suicide-MDD ^{41,143}		Alcohol ¹⁵⁷ 1q25.3 (linkage) Alcohol ¹⁶¹	4.0
<i>HOMER1</i> (homer homolog 1)	D	(D) MDD ¹³⁵ Fluoxetine ¹⁶² (D) Anxiety ¹⁶³ (D) Stress ¹⁶³ Alcohol HIP ¹¹⁶		(Transgenic) Abnormal response to addictive substance		(D) PTSD ⁴³	5q14.1 (association) MDD ¹⁶⁴	4.0
<i>PAM</i> (peptidylglycine α -amidating monooxygenase)	D	(I) DBP-ST PFC ²² (I) BP ¹⁶⁵		(QTL) Abnormal eating/drinking behavior; Addiction/drug abuse	(I) MDD ⁷⁹ (I) Suicide-MDD ¹⁵⁰	(D) Chronic stress ¹⁰⁴	5q21.1 (linkage) MDD ¹¹⁹ Alcohol ¹⁶⁶ BP ¹⁶⁷	4.0
<i>GABRB3</i> (γ -aminobutyric acid (GABA) A receptor, subunit β 3)	D	BP AMY, CP ²⁹ (D) DBP-ST AMY; (D) DBP-ST PFC ²²		(QTL) Addiction/drug abuse	(I) MDD ¹⁶⁸ (I) Alcohol ¹⁶⁹		15q12 (association) Alcohol ¹⁷⁰ BP ^{166,171}	3.5
<i>NCALD</i> (neurocalcin δ)	D	BP AMY, CP ²⁹		(QTL) Addiction/drug abuse	(I) Suicide-MDD ⁴¹		8q22.3 (association) Alcohol ¹⁷²	3.5
<i>OGT</i> (O-linked N-acetylglucosamine (GlcNAc) transferase)	D	(I) DBP-NST AMY ²²	DBP-ST BLOOD (D) ²²	(QTL) Abnormal emotion/affect behavior			Xq13.1 (association) BP ¹⁷³	3.5
<i>PTTG1</i> (pituitary tumor-transforming gene 1)	D	(D) DBP-NST AMY ²²				(I) PPD ⁸⁷	5q33.3 (linkage) BP ^{86,88,89} PD ⁹⁰	2.5

Abbreviations: AMY, amygdala; BP, bipolar; CFG, convergent functional genomics; CP, caudate putamen; D, decreased in expression; DBP, D-box binding protein; DHA, docosahexaenoic acid; HIP, hippocampus; I, increased in expression; KO, knockout; MDD, major depressive disorder; NAC, nucleus accumbens; NST, non-stressed; OCD, obsessive compulsive disorder; PD, panic disorder; PFC, prefrontal cortex; PPD, postpartum depression; PTSD, post-traumatic stress disorder; QTL, quantitative trait locus; ST, stressed; VT, ventral tegmentum. Myelin-related genes are underlined. Top candidate genes for which there were reproducible changes in expression in high-DHA vs low-DHA mice in PFC ($n = 7$), AMY ($n = 19$) and HIP ($n = 10$) are shown (CFG score of ≥ 3.5 points).

Table 2 Top gene expression changes in the brain in male DBP KO ST mice on high-DHA vs low-DHA diet

Gene symbol (name)	PFC change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>PTGDS</i> (prostaglandin D2 synthase)	I	(D) Anxiety ⁴⁹ (D) Stress ⁵⁰		(Transgenic) Decreased aggression	(D) Alcohol ¹¹⁴	(D) BP ⁴⁸	9q34.3 (association) Anxiety ¹⁷⁷ 12q13.12 (linkage) PD ¹⁷⁴	5.0
<i>ARF3</i> (ADP-ribosylation factor 3)	I	(D) DBP-ST AMY ²²		(QTL) Addiction/drug abuse	(D) Alcohol ¹¹⁴	(I) BP ⁷³ (D) Chronic stress ¹⁰⁴	1p31.3 (linkage) BP ^{61,82}	4.0
<i>NFIA</i> (nuclear factor I/A)	I	(I) DBP-NST AMY ²²		(QTL) Addiction/drug abuse Abnormal emotion/affect behavior	(I) MDD ⁷⁹	(I) Alcohol ⁸⁰	1p31.3 (linkage) BP ^{61,82}	4.0
<i>KLF4</i> (Kruppel-like factor 4)	I	Alcohol NAC ¹¹⁶ (D) Depression ¹⁰²		(Transgenic) Abnormal suckling behavior	(D) MDD ¹¹⁷		9q31.2 (linkage) BP ¹³² Anxiety/PD ¹³⁸	3.5
<i>PTTG1</i> (pituitary tumor-transforming gene 1)	D	(D) DBP-NST AMY ²²				(I) PPD ⁸⁷	5q33.3 (linkage) BP ^{86,88,89} PD ⁹⁰	2.5
Gene symbol (name)	AMY change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>AGT</i> (angiotensinogen)	I	BP NAC ²⁹ Alcohol CP, HIP, NAC, PFC ¹¹⁶ (D) DBP-NST AMY ²² (D) DBP-ST AMY ²²			(D) Alcohol ^{114,136}		1q42.2 (association) BP ¹⁷⁵	4.0
<i>HPCAL1</i> (hippocalcin-like 1)	I	(D) DBP-NST AMY ²²		(QTL) Abnormal circadian rhythm; addiction/drug abuse	(I) Suicide-MDD ⁴¹		2p25.1 (association) MDD ¹⁵¹	3.5
<i>PNOC</i> (prepronociceptin)	I	(D) DBP-NST AMY ²²		(QTL) Abnormal sleep pattern/circadian rhythm	(D) PTSD ¹⁷⁶		8p21.1 (association) Alcohol ¹⁷⁷	3.5
<i>SYT1</i> (synaptotagmin I)	D	(D) Depression ¹⁷⁸ (D) Alcohol ¹⁷⁹ BP AMY ²²⁹		(Transgenic) Abnormal suckling behavior	(D) Alcohol ¹⁸⁰ (D) BP ¹⁸¹		12q21.2 (linkage) BP ¹⁸² BP ¹⁸³ PD ¹⁸³ Alcohol ¹³⁷	3.5
<i>PER3</i> (period homolog 3)	D	(D) BP ¹¹³		(Transgenic) Shortened circadian period			1p36.23 (association) BP ¹⁸⁴ PD ¹⁸⁵ Alcohol ¹⁷⁰ Depression ¹⁸⁶	2.5
<i>PTTG1</i> (pituitary tumor-transforming gene 1)	D	(D) DBP-NST AMY ²²				(I) PPD ⁸⁷	5q33.3 (linkage) BP ^{86,88,89} PD ⁹⁰	2.5
Gene symbol (name)	HIP change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>FOS</i> (FBJ osteosarcoma oncogene)	I	(I) DBP-ST AMY ²² (I) Alcohol ⁸⁴ (D) MDD Fluoxetine ¹³⁵	BP ³⁰	(Transgenic) Decreased anxiety-related response	(I) BP ⁴²	(I) PTSD ⁴³ (I) Stress ⁴⁴	14q24.3 (linkage) BP ¹²⁰ Alcohol ¹⁶⁶	5.5

Table 2 (Continued)

Gene symbol (name)	HIP change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
DUSP1 (dual specificity phosphatase 1)	I	Alcohol AMY, CP, NAC, PFC ¹¹⁶ (I) Alcohol ⁸⁴	BP ³⁰	(QTL) Abnormal eating/drinking behavior; Abnormal circadian rhythm	(D) BP ¹⁸⁹ , MDD ³⁶	(I) Stress ⁴⁴	5q35.1 (linkage) PD ^{107,188}	5.0
GABRA1 (γ -aminobutyric acid (GABA) A receptor, subunit α 1)	I	(D) Stress ⁵⁰ (D) Alcohol ¹⁵⁴	BP ³⁰	(Transgenic) Impaired passive avoidance behavior	(I) BP ¹⁸⁹ (I) Suicide-MDD ⁴¹	(I) Mood ³⁰	5q34 (association) BP ^{45,46}	5.0
MBP (myelin basic protein)	I	(I) BP ¹¹³ (I) Anxiety ¹⁵²	BP ³⁰		(D) Suicide-MDD ⁴¹ (D) BP ⁵¹		18q23 (association) BP ¹⁹⁰	5.0
PTGDS (prostaglandin D2 synthase)	I	(D) Anxiety ⁴⁹ (D) Stress ⁵⁰		(Transgenic) Decreased aggression toward mice	(D) Alcohol ¹¹⁴	(D) BP ⁴⁸	9q34.3 (Association) Anxiety ⁴⁷	5.0
SPP1 (secreted phosphoprotein 1)	I	BP VT ²⁹	BP ³⁰	(QTL) Abnormal emotion/affect behavior Addiction/drug abuse	(D) Alcohol ¹⁸⁰ (D) Suicide-MDD ⁴¹ (D) MDD ³⁶	(D) Hallucinations ³¹	4q22.1 (linkage) Anxiety ⁷⁸ BP ¹²⁰	5.0
NR4A2 (nuclear receptor subfamily 4, group A, member 2)	I	(D) Anxiety ¹⁶² (D) Stress ⁵⁰		(Transgenic) Abnormal suckling behavior	(D) BP ¹⁹¹ , MDD ¹⁹¹	(D) Mood ³⁰	Alcohol ¹⁶¹ 2q24.1 (linkage) BP ⁸¹	4.5
CNP (β , β -cyclic nucleotide 3 phosphodiesterase)	I	(D) BP ¹¹³	BP ³⁰ DBP-NST Blood (D) ²²	(QTL) Addiction/drug abuse Abnormal emotion/affect behavior	(D) MDD ¹¹⁷ (D) Alcohol ^{114,136,192} (D) Suicide-MDD ⁴¹		17q21.2 (linkage) Alcohol ¹⁹³ BP ^{86,132}	4.0
GAD2 (glutamic acid decarboxylase 2)	I	(D) Alcohol ¹⁷⁹		(Transgenic) Decreased aggression	(D) BP ^{40,194,195}		10p12.1 (association) Alcohol ¹⁹⁶	4.0
GLS (glutaminase)	I			(QTL) Addiction/drug abuse	(D) BP ¹⁹⁸	(D) PTSD ⁴³	Anxiety/affective disorder ¹⁹⁷ 2q32.2 (linkage) Alcohol ^{166,199}	4.0
GLUL (glutamate-ammonia ligase) [†]	I	(I) Stress ¹⁵⁸ BP PFC, VT ²⁹ Alcohol AMY, NAC ¹¹⁶	BP ³⁰	(QTL) Abnormal emotion/affect behavior, Addiction/drug abuse	(D) MDD ^{159,160} (D) Suicide-MDD ^{41,143}		1q25.3 (linkage) Alcohol ¹⁶¹	4.0
HOMER1 (homer homolog 1)	I	(D) MDD-Fluoxetine ¹³⁵ (D) Anxiety ¹⁶² (D) Stress ¹⁶³ (D) Stress ¹¹⁶ Alcohol HIP ¹¹⁶		(Transgenic) Abnormal response to addictive substance	(D) PTSD ⁴³		5q14.1 (association) MDD ¹⁶⁴	4.0
NR3C2 (nuclear receptor subfamily 3, group C, member 2)	D	(D) Primate stress-induced ²⁰⁰		(Transgenic) Abnormal response to novel object	(I) MDD ¹³⁹		4q31.23 (association) Stress ²⁰¹	4.0
SLC12A2 (solute carrier family 12, member 2)	I	Alcohol HIP ¹¹⁶	BP ³⁰	(QTL) Abnormal eating/drinking behavior	(D) Alcohol ¹³⁶		5q23.2 (linkage) MDD ¹¹⁹	4.0
JAK1 (Janus kinase 1)	I	(D) Primate stress-induced ²⁰⁰		(Transgenic) Abnormal suckling behavior	(D) MDD ⁷⁹ (D) Alcohol ⁸⁵ (I) MDD ⁷⁹	(D) Stress ¹¹¹	1p31.3 (linkage) BP ^{81,82} Xq21.1	3.5
ATRX (α thalassemia/mental retardation syndrome X-linked)	I	(D) Anxiety ²⁰²	BP ³⁰	(Transgenic) Abnormal suckling behavior	(D) MDD ⁷⁹ (D) Alcohol ⁸⁵ (I) MDD ⁷⁹		10q22.3 (association) Alcohol ⁸³	3.5
KCNMA1 (potassium large conductance calcium-activated channel, subfamily M, α member 1)	I			Abnormal emotion/affect behavior, addiction/drug abuse				3.5

Table 2 (Continued)

Gene symbol (name)	HIP change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
<i>LPAR1</i> (lysophosphatidic acid receptor 1)	I	(D) MDD ¹¹⁷ (D) BP ¹¹³ (D) Depression ¹⁰²		(QTL) Abnormal sleep pattern/circadian rhythm; addiction/drug abuse	(D) MDD ¹¹⁷ (D) BP ²⁰³ (D) Suicide-MDD ⁴¹	(I) Mood ³⁰	9q31.3 (linkage) PD ^{138f} BP ¹⁵⁵	3.5
<i>MAPT</i> (microtubule-associated protein tau)	I	(D) Anxiety ²⁰⁴ Alcohol HIP ¹¹⁶		(Transgenic) Decreased anxiety-related response	(D) MDD ¹¹⁷ (I) Alcohol ¹⁸⁰		17q21.1 (linkage) Alcohol ¹⁹³ BP ^{36,152}	3.5
<i>MARCKS</i> (myristoylated alanine rich protein kinase C substrate)	I	(D) BP ^{113,205}		(Transgenic) Abnormal suckling behavior	(I) MDD ²⁰⁶		6q21 (linkage) BP ⁸¹	3.5
<i>MBNL1</i> (muscleblind-like 1)	I		BP ³⁰	(QTL) Abnormal emotion/affect behavior; Abnormal circadian rhythm	(I) MDD ⁷⁹		3q25.1 (association) BP ²⁰⁷	3.5
<i>NCALD</i> (neurocalcin δ)	I			(QTL) Addiction/drug abuse	(I) Suicide-MDD ⁴¹		8q22.3 (association) Alcohol ¹⁷²	3.5
<i>NRXN1</i> (neurexin 1)	I	(D) BP ¹¹³		(QTL) Addiction/drug abuse	(D) BP ²⁰⁸ (I) Suicide-MDD ⁴¹		2p16.3 (association) BP ¹⁷³ PD ²⁰⁹	3.5
<i>PIP4K2A</i> (phosphatidylinositol-5-phosphate 4-kinase, type II, α)	I	(D) Stress ⁵⁰ (D) Anxiety ²⁰⁴		(QTL) Addiction/drug abuse	(D) Alcohol ¹³⁶		10p12.2 (association) BP ²¹⁰	3.5
<i>PLXNA2</i> (plexin A2)	I			(QTL) Addiction/drug abuse	(I) Alcohol ¹³⁶	(D) Mood ³⁰	1q32.2 (association) Anxiety ²¹¹ BP ²⁰⁷	3.5
<i>SERPINI1</i> (serine (or cysteine) peptidase inhibitor, clade I, member 1)	I	(D) Primate stress-induced ²⁰⁰		(Transgenic) Abnormal anxiety-related response	(I) MDD ⁷⁹		3q26.1 (linkage) BP ^{81,212} PD ²¹³	3.5
<i>SNN</i> (stannin)	D	(D) Primate stress-induced ²⁰⁰		(QTL) Addiction/drug abuse	(I) Alcohol ¹³⁶		16p13.13 (linkage) Alcohol ²¹⁴ BP ⁹⁶	3.5
<i>PTTG1</i> (pituitary tumor-transforming gene 1)	D	(D) DBP-NST AMY ²²				(I) PPD ⁸⁷	5q33.3 (linkage) BP ^{36,88,89} PD ⁹⁰	2.5

Abbreviations: AMY, amygdala; BP, bipolar; CFG, convergent functional genomics; CP, caudate putamen; D, decreased in expression; DBP, *D*-box binding protein; DHA, docosahexaenoic acid; HIP, hippocampus; I, increased in expression; KO, knockout; MDD, major depressive disorder; NAC, nucleus accumbens; NST, non-stressed; OCD, obsessive compulsive disorder; PD, panic disorder; PFC, prefrontal cortex; PPD, postpartum depression; PTSD, post-traumatic stress disorder; QTL, quantitative trait locus; ST, stressed; VT, ventral tegmentum.

^aBlood biomarker. Top candidate genes for which there were reproducible changes in expression in high-DHA vs low-DHA mice in PFC ($n = 5$), AMY ($n = 5$) and HIP ($n = 29$) are shown (CFG score of ≥ 3.5 points). Myelin-related genes are underlined.

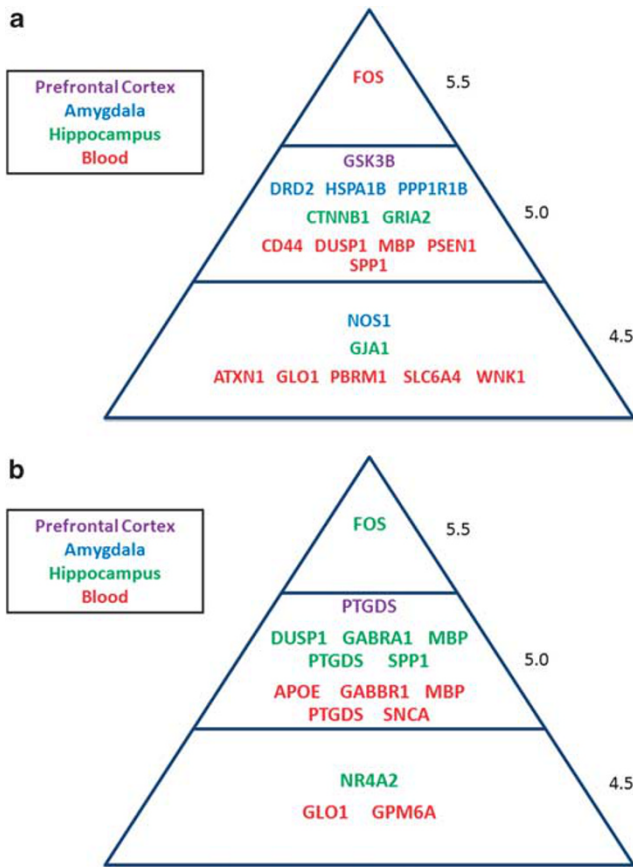


Figure 3 Top candidate genes changed in DBP knockout (KO) stressed (ST) mice on high- vs low-docosahexaenoic acid (DHA) diet. (a) Female mice and (b) male mice.

demonstrate such a powerful broad effect on myelin-related genes, and potentially reverse this pathology.

Sex differences and similarities at gene and pathway levels. There are profound sex differences, that is, there is little overlap, at individual gene levels, between the changes induced by DHA in males and in females. For example, in HIP, only five genes are changed in the same direction in males and females: *PTTG1* and *ADI1* (decreased by DHA) and *SCD*, *HBA-A1* and *HBB-B1* (increased by DHA). *PTTG1* (*pituitary tumor transforming gene 1*) is also decreased in all three male brain regions analyzed (PFC, AMY and HIP). *PTTG1* is an oncogene involved, among other things, in pituitary tumors. Its downregulation by DHA is indicative of potential anticancer benefits of DHA treatment that merit future exploration. However, at a pathway level, there is more overlap between males and females. For example, two of the five top five canonical pathways in HIP (glutamate receptor signaling, GABA receptor signaling) are shared between males and females, although different genes in these pathways are changed in each sex (Table 3b). Inflammation-related pathways are prominent in the PFC, and signaling pathways (cyclic adenosine monophosphate in females and circadian rhythm in males) in the AMY (Tables 3a and b).

Table 3 Ingenuity pathway analysis of the genes changed in DHA-treated mice: analysis of all differentially expressed genes in (a) female mice and (b) male mice

Pathways	P-value	Ratio
(a)		
<i>Top canonical pathways, female PFC (n = 66 genes)</i>		
Primary immunodeficiency signaling	2.59E-08	6/63 (0.095)
B-cell development	1.41E-07	5/37 (0.135)
Communication between innate and adaptive immune cells	2.49E-04	4/107 (0.037)
Autoimmune thyroid disease signaling	6.39E-04	3/61 (0.049)
Systemic lupus erythematosus signaling	1.52E-03	4/163 (0.025)
<i>Top canonical pathways, female AMY (n = 150 genes)</i>		
cAMP-mediated signaling	3.54E-07	10/161 (0.062)
G-protein-coupled receptor signaling	6.51E-07	11/222 (0.05)
Relaxin signaling	9.52E-06	8/151 (0.053)
Cardiac β -adrenergic signaling	4.27E-04	6/142 (0.042)
Protein kinase A signaling	5.61E-04	9/318 (0.028)
<i>Top canonical pathways, female HIP (n = 103 genes)</i>		
Glutamate receptor signaling	2.67E-04	4/70 (0.057)
Polyamine regulation in colon cancer	2.62E-03	2/22 (0.091)
GABA receptor signaling	2.49E-02	2/55 (0.036)
Mitotic roles of polo-like kinase	3.27E-02	2/62 (0.032)
TR/RXR activation	7.64E-02	2/99 (0.02)
(b)		
<i>Top canonical pathways, male PFC (n = 77 genes)</i>		
CCR5 signaling in macrophages	2.98E-03	3/93 (0.032)
Clathrin-mediated endocytosis signaling	2.52E-02	3/169 (0.018)
IL-8 signaling	3.04E-02	3/188 (0.016)
BMP signaling pathway	3.36E-02	2/80 (0.025)
Pathogenesis of multiple sclerosis	3.49E-02	1/9 (0.111)
<i>Top canonical pathways, male AMY (n = 59 genes)</i>		
Circadian rhythm signaling	2.16E-03	2/35 (0.057)
Neuroprotective role of THOP1 in Alzheimer's disease	4.16E-03	2/54 (0.037)
Glycine, serine and threonine metabolism	2.48E-02	2/150 (0.013)
Glycerophospholipid metabolism	4.55E-02	2/192 (0.01)
RAR activation	4.96E-02	2/181 (0.011)
<i>Top canonical pathways, male HIP (n = 352 genes)</i>		
Aldosterone signaling in epithelial cells	1.97E-05	9/97 (0.093)
Glutamate receptor signaling	7.12E-04	6/70 (0.086)
GABA receptor signaling	1.51E-03	5/55 (0.091)
RAR activation	2.22E-03	9/181 (0.05)
14-3-3-mediated signaling	2.89E-03	7/116 (0.06)

Abbreviations: AMY, amygdala; BMP, bone morphogenetic protein; cAMP, cyclic AMP; CCR5, chemokine (C-C motif) receptor 5; DHA, docosahexaenoic acid; GABA, γ -aminobutyric acid; HIP, hippocampus; IL, interleukin; PFC, prefrontal cortex; RAR, retinoic acid receptor; RXR, retinoid X receptor; THOP1, thimet oligopeptidase 1; TR, thyroid hormone receptor.

Circadian clock genes are also being modulated by DHA, with *PER3* (*period homolog 3*) being decreased in expression in the AMY of males, and *RORB* (*RAR-related orphan receptor β*) decreased in expression in PFC of females. Of note, we have previously reported evidence for genetic association of *RORB* with bipolar disorder in a pediatric bipolar cohort.⁵³

Blood biomarkers. *RAB27B* (from AMY), and *CAP1*, *CAPZB*, *GNG2*, *KLF9*, *NDUFS5*, *SSX2IP* and *VPS13A* (from HIP) are co-regulated in the same direction in brain and blood of DBP female mice by DHA (Table 4a). For male mice, *TFRC* (from PFC), *CD24A* and *FTL1* (from AMY), *GLUL*, *LIMD2*, *PSME4* and *TTR* (in HIP) are co-regulated in the same direction in brain and blood by DHA (Table 4b).

Table 4 Brain–blood concordant biomarkers modulated by DHA in (a) female mice and (b) male mice

(a) Females								
Gene symbol (name)	AMY and blood change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	
CFG score								
RAB27b (member RAS oncogene family)	D	(I) Alcohol ¹⁵⁴					18q21.2 (linkage) BP ¹⁴⁴ MDD ¹¹⁹	1.0
(b) Males								
Gene symbol (name)	HIP and blood change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	
CFG score								
KLf9 (Kruppel-like factor 9)	D	BP AMY, CP ²⁹ Alcohol NAC ¹¹⁶		(QTL) Abnormal circadian rhythm	(D) Alcohol ¹⁸⁰		9q21.12 (linkage) BP ^{147,215} Alcohol ^{137,199}	3.0
CAP1 (adenylate cyclase-associated protein 1)	D	(D) Depression ²¹⁶			(D) BP ²¹⁶ (D) MDD ¹¹⁷ (I) MDD ⁷⁹		1p34.2 (linkage) Alcohol ¹⁶¹ BP ²¹⁷	2.0
SSX2IP (synovial sarcoma, X breakpoint 2 interacting protein)	D				(D) Alcohol ¹⁸⁰		1p22.3 (linkage) BP ²¹⁷ Alcohol ^{134,199} Alcohol/depression ²¹⁸	1.5
NDUGA5 (NADH dehydrogenase (ubiquinone) Fe-S protein 5) VPS13A (vacuolar protein sorting 13A) CAPZB (capping protein (actin filament) muscle Z-line, β) GNG2 (guanine nucleotide binding protein (G protein), γ 2)	I D D D	Alcohol NAC ¹¹⁶ (I) DBP-ST PFC ²² (I) Depression ¹⁰² (I) Alcohol ¹⁵⁴						1.0 1.0 0.5 0.0
(b) Males								
Gene symbol (name)	PFC and blood change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	
CFG score								
TFFC (transferrin receptor)	I	Alcohol CP, HIP ¹¹⁶					3q29 (linkage) BP ²¹⁹ BP/SZ ²⁰ SZ, SZ, BP ¹⁴⁴ Alcohol ¹⁶¹	1.5
(a) Females								
Gene symbol (name)	AMY and blood change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	
CFG score								
CD24A (CD24a antigen) FTL1 (ferritin light chain 1)	I I	(D) DBP-ST AMY ²² BP ³⁰ (D) DBP-ST AMY; (D) DBP-ST PFC ²² Alcohol NAC ¹¹⁶						2.0 1.0
(b) Males								
Gene symbol (name)	HIP and blood change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	
CFG score								
GLUL (glutamate-ammmonia ligase)	I	(I) Stress ¹⁵⁸ BP PFC, VT ²⁹ Alcohol AMY, NAC ¹¹⁶	BP ³⁰	(QTL) Abnormal emotion/affect behavior, addiction/drug abuse	(D) MDD ^{159,180} (D) Suicide-behavior, addiction/drug MDD ^{41,145}		1q25.3 (linkage) Alcohol ¹⁶¹	4.0

Table 4 (Continued)

(b) Males		HIP and blood change	Animal brain evidence	Animal blood evidence	Animal genetic evidence	Human brain evidence	Human blood evidence	Human genetic evidence	CFG score
Gene symbol (name)									
LIMD2 (LIM domain containing 2)	D								1.0
TTR (transthyretin)	D		BP CP ²⁹ (I) Anxiety/ ¹⁴⁸ depression (I) Anxiety ^{49,142} (I) Depression ²²¹	BP ³⁰					1.0
PSME4 (proteasome (prosome, macropain) activator subunit 4)	I								0.0

Abbreviations: AMY, amygdala; BP, bipolar; CFG, convergent functional genomics; CP, caudate putamen; D, decreased in expression; DHA, docosahexaenoic acid; HIP, hippocampus; I, increased in expression; MDD, major depressive disorder; NAC, nucleus accumbens; NST, non-stressed; PFC, prefrontal cortex; PPD, postpartum depression; QTL, quantitative trait locus; ST, stressed; SZ, schizophrenia; SZA, schizoaffective disorder; VT, ventral tegmentum.

These genes warrant further studies in human clinical populations as potential gender-specific peripheral biomarkers of DHA treatment response.

In addition, a number of other genes are changed in expression by DHA in DBP mouse blood in opposite direction to that seen in human blood in mood disorders and stress disorders (Supplementary Tables S1 and S2). Although not changed in the same direction in the DBP mouse brain, at least in the limited numbers of regions we have assayed so far, they may nevertheless be viable human biomarkers of the therapeutic effects of DHA, upon further study and validation. Notably, one of these candidate markers is *SLC6A4* (solute carrier family 6 (neurotransmitter transporter, serotonin), member 4), decreased in expression by DHA in female DBP mouse blood.

Drugs that exert similar effects to DHA. A number of DHA-responsive genes identified by us in mice are modulated by existing drugs (Table 5), notably antipsychotics, benzodiazepines, calcium channel blockers and estrogens in females, respectively valproic acid and ketamine in males. Those classes of medications have a history of mood-modulating effects, use and abuse in bipolar and co-morbid disorders. Recent work has also shown that lithium can modulate DHA metabolism.⁵⁴

Effects of DHA on alcohol consumption in two independent animal models: DBP KO mice and alcohol-preferring P rats

DBP KO mice on high-DHA diet drink less alcohol than DBP KO mice on low DHA. The high rate of co-morbidity between bipolar disorder and alcoholism in humans⁵⁵ is reflected in our DBP KO mice animal model. We had previously shown that male DBP KO mice subjected to the chronic isolation stress paradigm consume more ethanol than the control DBP WT mice subjected to stress.²² We have now tested if a high-DHA diet would impact the alcohol consumption of these DBP KO mice compared with a low-DHA diet. In two separate analyses, one from a 2-week experiment and one from a 4-week experiment, we found that DHA significantly reduces alcohol consumption (Figure 4). No significant differences in water consumption were observed (data not shown), which shows that mice are showing a preference for alcohol, and not simply drinking more fluids.

P rats on high-DHA diet drink less alcohol than P rats on low-DHA diet. We were able to reproduce our findings in a well-established, independent animal model of alcohol consumption, the alcohol-preferring P rats. These rats are also subjected to single housing, which may induce chronic stress. Additionally, for these experiments, we did not just look at extremes of diet in terms of DHA content, but also used a normal control diet, with an intermediate content of DHA. A dose-dependent effect was observed, where alcohol-preferring P rats on a diet high in DHA drank significantly less alcohol over a 14-day period than did P rats on a normal control diet, and rats on a diet low in DHA (Figure 5).

Table 5 DHA-responsive genes in our data set that are the targets of existing drugs**(a) Females**

Gene symbol (name)	Type(s)	Drug(s)	CFG score
PFC			
<i>GSK3B</i> (glycogen synthase kinase 3 β)	Kinase	Enzastaurin	5.0
<i>KCNMA1</i> (potassium large conductance calcium-activated channel, subfamily M, α member 1)	Ion channel	Tedisamil	3.5
AMY			
<i>DRD2</i> (dopamine receptor D2)	G-protein-coupled receptor	Paliperidone, risperidone, buspirone, bifeprunox, iloperidone, blonanserin, asenapine, pardoprinox, ocaperidone, abaperidone, SLV-314, RGH-188, rotigotine, opipramol, chlorpromazine, metoclopramide, sulpiride, meloxicam, amantadine, trifluoperazine, fluphenazine, pimozide, clozapine, haloperidol, fluoxetine/olanzapine, fluphenazine decanoate, thiothixene, amitriptyline/perphenazine, haloperidol decanoate, molindone, trimethobenzamide	5.0
<i>NOS1</i> (nitric oxide synthase 1) (neuronal)	Enzyme	GW 273629, omega-N-methylarginine	4.5
<i>ALDH1A1</i> (aldehyde dehydrogenase 1 family, member A1)	Enzyme	Disulfiram, chlorpropamide	3.5
<i>ESR1</i> (estrogen receptor 1)	Ligand-dependent nuclear receptor	17- α -ethinylestradiol, fulvestrant, β -estradiol, estradiol 17- β -cypionate, estrone, estradiol valerate, 3-(4-methoxy)phenyl-4-((4-(2-(1-piperidinyl)ethoxy)phenyl)methyl)-2H-1-benzopyran-7-ol, bazedoxifene, estradiol valerate/testosterone enanthate, TAS-108, ethinyl estradiol/ethynodiol diacetate, estradiol acetate, esterified estrogens, estradiol cypionate/medroxyprogesterone acetate, conjugated estrogens/meprobamate, estradiol/norethindrone acetate, synthetic conjugated estrogens	3.5
<i>GABRD</i> (γ -aminobutyric acid (GABA) A receptor, δ)	Ion channel	Pagoclone, alphadolone, SEP 174559, tracazolate, sevoflurane, isoflurane, gaboxadol, felbamate, etomidate, muscimol, halothane, fluoxetine/olanzapine, eszopiclone, temazepam, zolpidem, lorazepam, olanzapine, clonazepam, zaleplon, secobarbital, phenobarbital, pentobarbital, D 23129, desflurane, methoxyflurane, enflurane, pregnenolone	3.5
<i>PDE7B</i> (phosphodiesterase 7B)	Enzyme	Dyphylline, nitroglycerin, aminophylline, anagrelide, milrinone, dipyridamole, tolbutamide, theophylline, pentoxifylline	1.0
<i>SCN4B</i> (sodium channel, voltage-gated, type IV, β)	Ion channel	Riluzole	1.0
HIP			
<i>GRIA2</i> (glutamate receptor, ionotropic, AMPA 2)	Ion channel	Talampanel, Org 24448, LY451395, tezampanel	5.0
<i>GABRB3</i> (γ -aminobutyric acid (GABA) A receptor, β 3)	Ion channel	Methohexital, aspirin/butalbital/caffeine, aspirin/butalbital/caffeine/codeine, pagoclone, alphadolone, SEP 174559, acetaminophen/butalbital/caffeine, sevoflurane, isoflurane, gaboxadol, isoniazid, felbamate, etomidate, muscimol, halothane, fluoxetine/olanzapine, amobarbital, atropine/hyoscyamine/phenobarbital/scopolamine, acetaminophen/butalbital, eszopiclone, mephobarbital, hyoscyamine/phenobarbital, acetaminophen/butalbital/caffeine/codeine, butabarbital, temazepam, zolpidem, lorazepam, olanzapine, clonazepam, zaleplon, secobarbital, butalbital, phenobarbital, pentobarbital, thiopental, D 23129, desflurane, methoxyflurane, enflurane, pregnenolone	4.5
<i>CACNA2D1</i> (calcium channel, voltage-dependent, α 2/ δ subunit 1)	Ion channel	Amlodipine/valsartan/hydrochlorothiazide, amlodipine/telmisartan, bepridil, amlodipine, pregabalin	1.0
<i>IFNGR2</i> (interferon γ receptor 2 (interferon γ transducer 1))	Transmembrane receptor	Interferon γ -1b	1.0

(b) Males

PFC			
<i>COL6A2</i> (collagen, type VI, α 2)	Other	Collagenase clostridium histolyticum	1.0
<i>CCR5</i> (chemokine (C-C motif) receptor 5)	G-protein-coupled receptor	Maraviroc, vicriviroc, SCH 351125	0.5
AMY			
<i>GRIN2C</i> (glutamate receptor, ionotropic, N-methyl D-aspartate 2C)	Ion channel	Dextromethorphan/guaifenesin, morphine/dextromethorphan, neramexane, bicifadine, delucemine, CR 2249, besonprodil, UK-240455, ketamine, felbamate, memantine, orphenadrine, cycloserine, N-(2-indanyl)glycinamide, dextromethorphan	2.0

Table 5 (Continued)

(b) Males

Gene symbol (name)	Type(s)	Drug(s)	CFG score
<i>HIP</i> <i>GABRA1</i> (γ -aminobutyric acid (GABA) A receptor, α 1)	Ion channel	Methohexital, aspirin/butalbital/caffeine, aspirin/butalbital/caffeine/codeine, pagoclone, alphadolone, SEP 174559, acetaminophen/butalbital/caffeine, sevoflurane, isoflurane, gaboxadol, isoniazid, felbamate, etomidate, muscimol, halothane, fluoxetine/olanzapine, amobarbital, estazolam	5.0
<i>GAD2</i> (glutamate decarboxylase 2)	Enzyme	Valproic acid	4.0
<i>NR3C2</i> (nuclear receptor subfamily 3, group C, member 2)	Ligand-dependent nuclear receptor	Hydrochlorothiazide/spironolactone, fludrocortisone acetate, drospirenone, spironolactone, eplerenone	4.0
<i>SLC12A2</i> (solute carrier family 12 (sodium/potassium/chloride transporters), member 2)	Transporter	Bumetanide	4.0
<i>KCNMA1</i> potassium large conductance calcium-activated channel, subfamily M, α member 1	Ion channel	Tedisamil	3.5
<i>ATP1A2</i> (ATPase, Na ⁺ /K ⁺ transporting, α 2 polypeptide)	Transporter	Digoxin, omeprazole, ethacrynic acid, perphenazine	2.5
<i>LPL</i> (lipoprotein lipase)	Enzyme	Nicotinic acid, lovastatin/niacin	2.0
<i>SLC1A3</i> (solute carrier family 1 (glial high affinity glutamate transporter), member 3)	Transporter	Riluzole	2.0
<i>SLC6A1</i> (solute carrier family 6 (neurotransmitter transporter, GABA), member 1)	Transporter	Tiagabine	2.0
<i>CHUK</i> (conserved helix-loop-helix ubiquitous kinase)	Kinase	Methyl 2-cyano-3,12-dioxoolean-1,9-dien-28-oate	1.0
<i>PARP1</i> (poly (ADP-ribose) polymerase 1)	Enzyme	ABT-888, INO-1001	1.0
<i>SCN1A</i> (sodium channel, voltage-gated, type I, α subunit)	Ion channel	Articaine/epinephrine, articaine, bupivacaine/lidocaine, chloroprocaine, epinephrine/prilocaine, epinephrine/lidocaine, fosphenytoin, phenytoin, prilocaine, lamotrigine, lidocaine, riluzole	1.0
<i>TGFB2</i> (transforming growth factor, β 2)	Growth factor	AP-12009	1.0
<i>HTR5A</i> (5-hydroxytryptamine (serotonin) receptor 5A)	G-protein-coupled receptor	Asenapine	0.5
<i>SCN8A</i> (sodium channel, voltage gated, type VIII, α subunit)	Ion channel	Riluzole	0.5

Abbreviations: AMY, amygdala; CFG, convergent functional genomics; DHA, docosahexaenoic acid; HIP, hippocampus; PFC, prefrontal cortex. Ingenuity analyses of the genes that are targeted by existing drugs.

Discussion

We conducted integrative studies of DHA treatment in animal models as a way of validating the efficacy of DHA as a psychotropic agent, to understand its underlying molecular effects in the brain, and to identify potential blood biomarkers of treatment response. Our work provides evidence on all three counts. Moreover, it identifies a previously unsuspected effect of DHA on decreasing alcohol consumption, which we substantiated in two independent animal models.

DBP KO ST mice as a human disease-relevant animal model. First, the behavioral phenomenology and inferences from molecular changes in the DBP KO mice revealed by our previous work²² bear broad similarities to the DSM (Diagnostic and Statistical Manual of Mental Disorders) criteria for bipolar disorder. Moreover, their switch in phenotype is a cardinal aspect of the human condition. As such, DBP KO mice are arguably one of the first comprehensive genetic animal models of bipolar disorder to be described, complementing earlier elegant pharmacological and genetic manipulations that mimic more

restricted endophenotypic aspects of the disorder.^{19,29,56–65} The fact that DBP is a transcription factor directly and indirectly regulating many other genes may explain the surprisingly comprehensive mimicry of a putative polygenic human disorder by a single gene ablation in mouse. Some of the genes identified may be directly regulated by DBP through promoter binding, whereas others may be regulated indirectly by a cascade of gene expression changes set in motion by DBP. Moreover, DBP is a circadian clock regulator, and an emerging body of work^{53,66–68} substantiates the role of clock genes in bipolar and related disorders.

The DBP KO mice are a constitutive KO, and there is always the possibility that compensatory changes can occur during development that may obscure the direct effects of DBP deletion. However, of note, this is a very good equivalent of the human bipolar disorder genetic scenario, where most mutations are likely constitutive rather than acquired, as reflected in the familial inheritance of the disorder. Second, our mice colony is on a mixed genetic background, generated by heterozygote breeding, not on a back-crossed pure mouse-strain background. Although this introduces epistatic

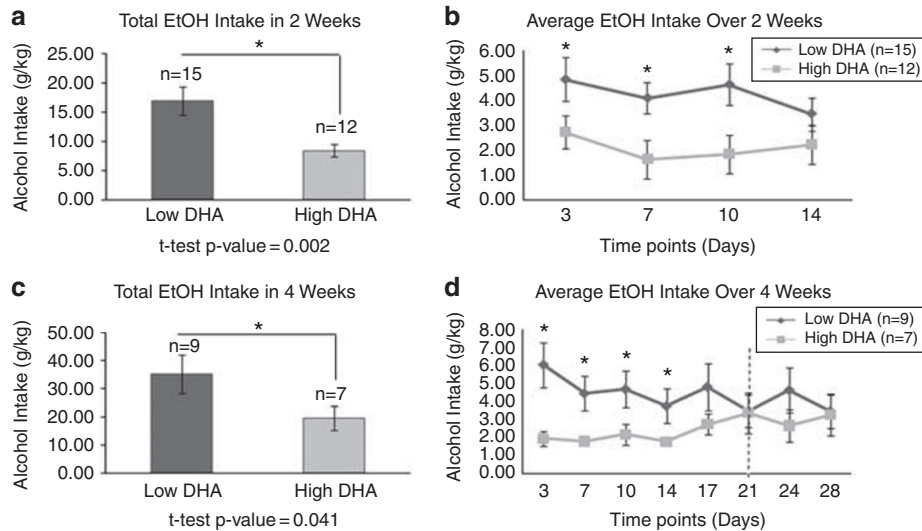


Figure 4 Effects of docosahexaenoic acid (DHA) on male DBP knockout (KO) stressed (ST) mice alcohol (EtOH) consumption: mice on a diet supplemented with either low or high DHA were subjected to alcohol free-choice drinking paradigm. (a, b) Fluid consumption (water or 10% ethanol) monitored for a period of 2 weeks (14 days). (c, d) Fluid consumption (water or 10% ethanol) monitored for a period of 4 weeks (28 days) with an acute stressor (behavioral challenge tests represented by the dotted vertical line) at day 21, as described in the Materials and methods Section. $*P < 0.05$.

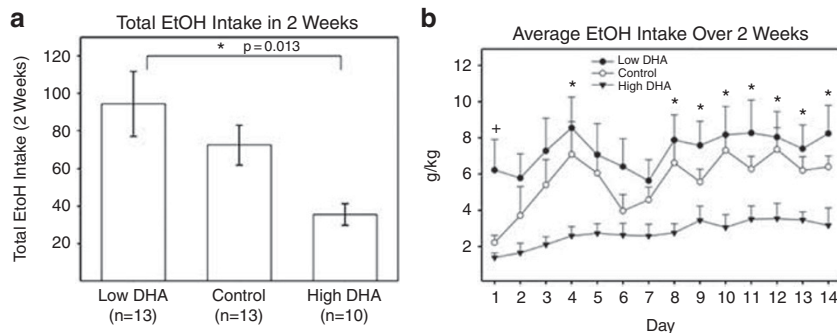


Figure 5 Effects of docosahexaenoic acid (DHA) on alcohol (EtOH) consumption in male alcohol-preferring (P) rats. Experimentally naive, male P rats, 4–6 months of age at the start of the experiment, were used as subjects. These rats were placed on one of the three diets: (1) low-DHA diet, (2) control diet or (3) high-DHA diet. Rats were given continuous free-choice access in the home cage to 15% v/v ethanol and water. Ethanol intake was measured daily throughout the experiment. (a, b) Fluid consumption from both bottles was monitored for a period of 2 weeks (14 days). $*t$ -test $P < 0.05$ for rats on low-DHA compared with rats on high-DHA diet.

variability, it is remarkable that the phenotype remains penetrant across generations and cohorts of mice. Again, however, this is a better model of the human condition, which occurs at a population level in a mixed genetic background, than deriving conclusions from a very particular strain of mice.

Stress is an important trigger of medical and mental illness episodes in humans. Acute overwhelming stress (accidents, illness, loss of employment) on top of the chronic stress of social isolation often precede decompensation in bipolar patients⁶⁹ and relapse into alcoholism.⁷⁰ With that in mind, our mice were subjected to a chronic stress paradigm consisting of isolation (single housing) for 1 month, overlaid with an acute stressor (a series of behavioral challenge tests) at the end of the third week of isolation.

Last, the insights into overlapping phenomics, genomics and biomarkers among bipolar, alcoholism, stress and related disorders provided by this mouse model recapitulates in a translational fashion to the issues of complexity, heterogene-

ity, overlap and interdependence of major psychiatric syndromes as currently defined by DSM⁷¹ that are seen in human patients.

The power of the CFG approach. By cross-validating our animal model gene expression data with other lines of evidence, including human data, we were able to extract a shorter list of genes for which there are external corroborating lines of evidence (human genetic evidence, human post-mortem brain data, human blood data, animal model QTL data) linking them to bipolar and related disorders, thus reducing the risk of false positives. This cross-validation also identifies candidate blood biomarkers that are more likely directly related to the relevant disease neuropathology, as opposed to being potential artifactual effects related to a particular animal cohort or indirect effects of mouse colony environment. The power of our CFG approach is exemplified in the fact that our biomarker

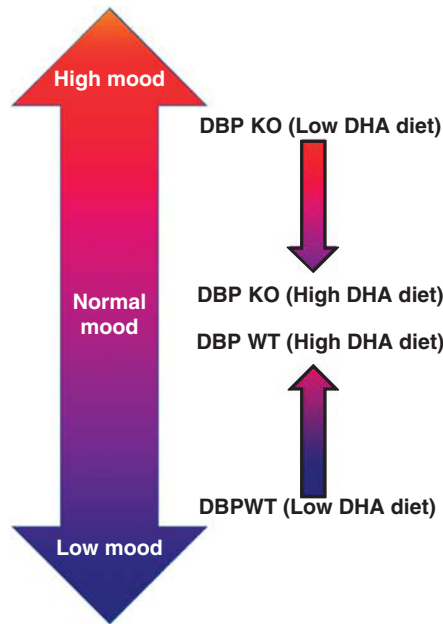


Figure 6 High-docosahexaenoic acid (DHA) diet has a stabilizing effect on mood in stressed mice. A model integrating the behavioral and genomic data.

findings from previous studies have been shown to have good predictive power in independent cohorts,^{30,31} a key litmus test in our view, and one that needs to be applied more systematically in this nascent field. The concordant candidate blood biomarkers for response to DHA that we identified in the current study (notably *GLUL* (*glutamate-ammonia ligase glutamine synthetase*) in males and *KLF9* (*Kruppel-like factor 9*) in females), as well as some of the blood-only candidates that are changed in reverse direction to that seen in human blood in mood and stress disorders (notably *SLC6A4* in females, as well as *MBP* and *GLO1* in both sexes), will need to pass that level of scrutiny in future human studies before being deemed of unambiguous value.

From genes and biomarkers to biology. There is little co-directional overlap between the DHA-modulated genes in females and in males identified by us, which is somewhat surprising and quite interesting. However, there is some overlap at a biological pathway level and behavioral level between males and females. A practical implication of this work would be the need to use gender-specific biomarkers of response to treatment. Overall, the model that is emergent from the behavioral and gene expression data is that of DHA acting as a mood-stabilizing agent (Figure 6).

Future studies by us and others may focus on understanding at a mechanistic level the novel uncovered effects on alcohol consumption. We also need to test for potential gender differences in the effects of DHA on alcohol consumption.

Conclusions. Taken together, our convergent results provide evidence that DHA modulates and is involved in molecular networks targeted by current psychotropic medications. They also suggest intriguing possible sex

differences for the molecular and behavioral effects of DHA, with a more antidepressant-like profile in females and a more antimanic-like profile in males.

The overall case for using DHA in large-scale human clinical trials and empirical clinical practice as an adjuvant mood-stabilizing agent and a novel potential alcoholism treatment, particularly for co-morbid bipolar disorder and alcoholism, is suggested and beginning to be substantiated at a mechanistic level by our work. Other possible therapeutic effects of DHA (in psychosis, anxiety, stress, pain and substance abuse) are pointed at by some of our data, and existing data in the literature. Given the genetic and biological heterogeneity of psychiatric disorders in human populations, it is possible, indeed likely, that not everyone will respond equally well to DHA treatment. Gender distinctions may be important, as our work suggests. The candidate blood biomarkers identified by us merit hypothesis-driven follow-up studies as markers of treatment response in a clinical setting; i.e., to test whether they are able to stratify, predict and differentiate early on in treatment responders from nonresponders.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. This work was supported by funds from the Indiana University, NARSAD, a VA Merit Award and a NIH Directors' New Innovator Award to ABN.

1. Cunnane SC, Crawford MA. Survival of the fattest: fat babies were the key to evolution of the large human brain. *Comp Biochem Physiol A Mol Integr Physiol* 2003; **136**: 17–26.
2. Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother* 2006; **60**: 502–507.
3. DeMar Jr JC, Ma K, Bell JM, Igarashi M, Greenstein D, Rapoport SI. One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores in rats. *J Lipid Res* 2006; **47**: 172–180.
4. Rao JS, Ertley RN, Lee HJ, DeMar Jr JC, Arnold JT, Rapoport SI *et al*. n-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism. *Mol Psychiatry* 2007; **12**: 36–46.
5. Zanarini MC, Frankenburg FR. Omega-3 Fatty acid treatment of women with borderline personality disorder: a double-blind, placebo-controlled pilot study. *Am J Psychiatry* 2003; **160**: 167–169.
6. Lin PY, Huang SY, Su KP. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biol Psychiatry* 2010; **68**: 140–147.
7. McNamara RK. DHA deficiency and prefrontal cortex neuropathology in recurrent affective disorders. *J Nutr* 2010; **140**: 864–868.
8. McNamara RK, Jandacek R, Rider T, Tso P, Stanford KE, Hahn CG *et al*. Deficits in docosahexaenoic acid and associated elevations in the metabolism of arachidonic acid and saturated fatty acids in the postmortem orbitofrontal cortex of patients with bipolar disorder. *Psychiatry Res* 2008; **160**: 285–299.
9. Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E *et al*. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch Gen Psychiatry* 1999; **56**: 407–412.
10. Parker G, Gibson NA, Brotchie H, Heruc G, Rees AM, Hadzi-Pavlovic D. Omega-3 fatty acids and mood disorders. *Am J Psychiatry* 2006; **163**: 969–978.
11. Osher Y, Belmaker RH. Omega-3 fatty acids in depression: a review of three studies. *CNS Neurosci Ther* 2009; **15**: 128–133.
12. Clayton EH, Hanstock TL, Himeth SJ, Kable CJ, Garg ML, Hazell PL. Reduced mania and depression in juvenile bipolar disorder associated with long-chain omega-3 polyunsaturated fatty acid supplementation. *Eur J Clin Nutr* 2009; **63**: 1037–1040.
13. Peet M, Stokes C. Omega-3 fatty acids in the treatment of psychiatric disorders. *Drugs* 2005; **65**: 1051–1059.
14. Berger GE, Wood SJ, Wellard RM, Proffitt TM, McConchie M, Amminger GP *et al*. Ethyl-eicosapentaenoic acid in first-episode psychosis. A 1H-MRS study. *Neuropsychopharmacology* 2008; **33**: 2467–2473.

15. Amminger GP, Schafer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM *et al*. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. *Arch Gen Psychiatry* 2010; **67**: 146–154.
16. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA* 2010; **303**: 250–257.
17. Muldoon MF, Ryan CM, Sheu L, Yao JK, Conklin SM, Manuck SB. Serum phospholipid docosahexaenoic acid is associated with cognitive functioning during middle adulthood. *J Nutr* 2010; **140**: 848–853.
18. Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L *et al*. Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels. *J Neurosci* 2007; **27**: 4385–4395.
19. Niculescu III AB, Segal DS, Kuczenski R, Barrett T, Hauger RL, Kelsoe JR. Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. *Physiol Genomics* 2000; **4**: 83–91.
20. Rodd ZA, Bertsch BA, Strother WN, Le-Niculescu H, Balaraman Y, Hayden E *et al*. Candidate genes, pathways and mechanisms for alcoholism: an expanded convergent functional genomics approach. *Pharmacogenomics J* 2007; **7**: 222–256.
21. Le-Niculescu H, Balaraman Y, Patel S, Tan J, Sidhu K, Jerome RE *et al*. Towards understanding the schizophrenia code: an expanded convergent functional genomics approach. *Am J Med Genet B Neuropsychiatr Genet* 2007; **144**: 129–158.
22. Le-Niculescu H, McFarland M, Ogden C, Balaraman Y, Patel S, Tan J *et al*. Phenomic, convergent functional genomic, and biomarker studies in a stress-reactive genetic animal model of bipolar disorder and co-morbid alcoholism. *Am J Med Genet B* 2008; **147B**: 134–166.
23. Niculescu AB, Le-Niculescu H. The P-value illusion: how to improve (psychiatric) genetic studies. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 847–849.
24. Schuckit MA, Smith TL, Chacko Y. Evaluation of a depression-related model of alcohol problems in 430 probands from the San Diego prospective study. *Drug Alcohol Depend* 2006; **82**: 194–203.
25. Kuo PH, Gardner CO, Kendler KS, Prescott CA. The temporal relationship of the onsets of alcohol dependence and major depression: using a genetically informative study design. *Psychol Med* 2006; **36**: 1153–1162.
26. Numberger Jr JI, Wiegand R, Bucholz K, O'Connor S, Meyer ET, Reich T *et al*. A family study of alcohol dependence: coaggregation of multiple disorders in relatives of alcohol-dependent probands. *Arch Gen Psychiatry* 2004; **61**: 1246–1256.
27. Lim GP, Calon F, Morihiro T, Yang F, Teter B, Ubeda O *et al*. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J Neurosci* 2005; **25**: 3032–3040.
28. Niculescu AB, Lulow LL, Ogden CA, Le-Niculescu H, Salomon DR, Schork NJ *et al*. PhenoChipping of psychotic disorders: a novel approach for deconstructing and quantitating psychiatric phenotypes. *Am J Med Genet B Neuropsychiatr Genet* 2006; **141**: 653–662.
29. Ogden CA, Rich ME, Schork NJ, Paulus MP, Geyer MA, Lohr JB *et al*. Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol Psychiatry* 2004; **9**: 1007–1029.
30. Le-Niculescu H, Kurian SM, Yehyawi N, Dike C, Patel SD, Edenberg HJ *et al*. Identifying blood biomarkers for mood disorders using convergent functional genomics. *Mol Psychiatry* 2009; **14**: 156–174.
31. Kurian SM, Le-Niculescu H, Patel SD, Bertram D, Davis J, Dike C *et al*. Identification of blood biomarkers for psychosis using convergent functional genomics. *Mol Psychiatry* 2011; **16**: 37–58.
32. Monteggia LM, Luikart B, Barrot M, Theobald D, Malkovska I, Nef S *et al*. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry* 2007; **61**: 187–197.
33. Niculescu AB, Akiskal HS. Sex hormones, Darwinism, and depression. *Arch Gen Psychiatry* 2001; **58**: 1083–1084; author reply 5–6.
34. Niculescu III AB, Akiskal HS. Proposed endophenotypes of dysthymia: evolutionary, clinical and pharmacogenomic considerations. *Mol Psychiatry* 2001; **6**: 363–366.
35. Patel SD, Le-Niculescu H, Koller DL, Green SD, Lahiri DK, McMahon FJ *et al*. Coming to grips with complex disorders: genetic risk prediction in bipolar disorder using panels of genes identified through convergent functional genomics. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 850–877.
36. Vawter MP, Tomita H, Meng F, Bolstad B, Li J, Evans S *et al*. Mitochondrial-related gene expression changes are sensitive to agonist-pH state: implications for brain disorders. *Mol Psychiatry* 2006; **11**: 615, 663–679.
37. O'Brien WT, Klein PS. Validating GSK3 as an in vivo target of lithium action. *Biochem Soc Trans* 2009; **37**(Pt 5): 1133–1138.
38. Svenningsson P, Tzavara ET, Carruthers R, Rachleff I, Wattler S, Nehls M *et al*. Diverse psychotomimetics act through a common signaling pathway. *Science* 2003; **302**: 1412–1415.
39. Perlis RH, Smoller JW, Ferreira MA, McQuillin A, Bass N, Lawrence J *et al*. A genomewide association study of response to lithium for prevention of recurrence in bipolar disorder. *Am J Psychiatry* 2009; **166**: 718–725.
40. Benes FM, Lim B, Matzilevich D, Subburaj S, Walsh JP. Circuitry-based gene expression profiles in GABA cells of the trisynaptic pathway in schizophrenics versus bipolars. *Proc Natl Acad Sci USA* 2008; **105**: 20935–20940.
41. Sequeira A, Mamdani F, Ernst C, Vawter MP, Bunney WE, Lebel V *et al*. Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. *PLoS One* 2009; **4**: e6585.
42. Rao JS, Harry GJ, Rapoport SI, Kim HW. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. *Mol Psychiatry* 2010; **15**: 384–392.
43. Segman RH, Shefi N, Goltser-Dubner T, Friedman N, Kaminski N, Shalev AY. Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Mol Psychiatry* 2005; **10**: 500–513, 425.
44. Morita K, Saito T, Ohta M, Ohmori T, Kawai K, Teshima-Kondo S *et al*. Expression analysis of psychological stress-associated genes in peripheral blood leukocytes. *Neurosci Lett* 2005; **381**: 57–62.
45. Horiuchi Y, Nakayama J, Ishiguro H, Ohtsuki T, Detera-Wadleigh SD, Toyota T *et al*. Possible association between a haplotype of the GABA-A receptor alpha 1 subunit gene (GABRA1) and mood disorders. *Biol Psychiatry* 2004; **55**: 40–45.
46. Breuer R, Hamshere ML, Strohmaier J, Mattheisen M, Degenhardt F, Meier S *et al*. Independent evidence for the selective influence of GABA(A) receptors on one component of the bipolar disorder phenotype. *Mol Psychiatry*; advance online publication, 15 June 2010 [e-pub ahead of print].
47. Donner J, Pirkola S, Silander K, Kananen L, Terwilliger JD, Lonnqvist J *et al*. An association analysis of murine anxiety genes in humans implicates novel candidate genes for anxiety disorders. *Biol Psychiatry* 2008; **64**: 672–680.
48. Begemann M, Sargin D, Rossner MJ, Bartels C, Theis F, Wichert SP *et al*. Episode-specific differential gene expression of peripheral blood mononuclear cells in rapid cycling supports novel treatment approaches. *Mol Med* 2008; **14**: 546–552.
49. Lee KW, Lee SH, Kim H, Song JS, Yang SD, Paik SG *et al*. Progressive cognitive impairment and anxiety induction in the absence of plaque deposition in C57BL/6 inbred mice expressing transgenic amyloid precursor protein. *J Neurosci Res* 2004; **76**: 572–580.
50. Reyes TM, Walker JR, DeCino C, Hogenesch JB, Sawchenko PE. Categorically distinct acute stressors elicit dissimilar transcriptional profiles in the paraventricular nucleus of the hypothalamus. *J Neurosci* 2003; **23**: 5607–5616.
51. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB *et al*. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 2003; **362**: 798–805.
52. Haroutunian V, Katsel P, Dracheva S, Stewart DG, Davis KL. Variations in oligodendrocyte-related gene expression across multiple cortical regions: implications for the pathophysiology of schizophrenia. *Int J Neuropsychopharmacol* 2007; **10**: 565–573.
53. McGrath CL, Glatt SJ, Sklar P, Le-Niculescu H, Kuczenski R, Doyle AE *et al*. Evidence for genetic association of RORB with bipolar disorder. *BMC Psychiatry* 2009; **9**: 70.
54. Basselin M, Kim HW, Chen M, Ma K, Rapoport SI, Murphy RC *et al*. Lithium modifies brain arachidonic and docosahexaenoic metabolism in rat lipopolysaccharide model of neuroinflammation. *J Lipid Res* 2010; **51**: 1049–1056.
55. Ostacher MJ, Perlis RH, Nierenberg AA, Calabrese J, Stange JP, Salloum I *et al*. Impact of substance use disorders on recovery from episodes of depression in bipolar disorder patients: prospective data from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). *Am J Psychiatry* 2010; **167**: 289–297.
56. Shalubina A, Einat H, Szechtman H, Shimon H, Belmaker RH. Preliminary evaluation of oral anticonvulsant treatment in the quinpirole model of bipolar disorder. *J Neural Transm* 2002; **109**: 433–440.
57. Gould TD, Einat H, Bhat R, Manji HK. AR-A014418, a selective GSK-3 inhibitor, produces antidepressant-like effects in the forced swim test. *Int J Neuropsychopharmacol* 2004; **7**: 387–390.
58. Einat H, Manji HK, Gould TD, Du J, Chen G. Possible involvement of the ERK signaling cascade in bipolar disorder: behavioral leads from the study of mutant mice. *Drug News Perspect* 2003; **16**: 453–463.
59. Einat H, Manji HK. Cellular plasticity cascades: genes-to-behavior pathways in animal models of bipolar disorder. *Biol Psychiatry* 2006; **59**: 1160–1171.
60. Einat H. Establishment of a battery of simple models for facets of bipolar disorder: a practical approach to achieve increased validity, better screening and possible insights into endophenotypes of disease. *Behav Genet* 2007; **37**: 244–255.
61. Roybal K, Theobald D, Graham A, DiNieri JA, Russo SJ, Krishnan V *et al*. Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci USA* 2007; **104**: 6406–6411.
62. Chen G, Henter ID, Manji HK. Translational research in bipolar disorder: emerging insights from genetically based models. *Mol Psychiatry* 2010; **15**: 883–895.
63. Gould TD, O'Donnell KC, Picchini AM, Dow ER, Chen G, Manji HK. Generation and behavioral characterization of beta-catenin forebrain-specific conditional knock-out mice. *Behav Brain Res* 2008; **189**: 117–125.
64. Kubota M, Kasahara T, Iwamoto K, Komori A, Ishiwata M, Miyauchi T *et al*. Therapeutic implications of down-regulation of cyclophilin D in bipolar disorder. *Int J Neuropsychopharmacol* 2010; **13**: 1355–1368.
65. Kasahara T, Kubota M, Miyauchi T, Noda Y, Mouri A, Nabeshima T *et al*. Mice with neuron-specific accumulation of mitochondrial DNA mutations show mood disorder-like phenotypes. *Mol Psychiatry* 2006; **11**: 577–593, 523.

66. Lavebratt C, Sjöholm LK, Partonen T, Schalling M, Forsell Y. PER2 variation is associated with depression vulnerability. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 570–581.
67. Lavebratt C, Sjöholm LK, Soronen P, Paunio T, Vawter MP, Bunney WE et al. CRY2 is associated with depression. *PLoS One* 2010; **5**: e9407.
68. Sjöholm LK, Kovanen L, Saarikoski ST, Schalling M, Lavebratt C, Partonen T. CLOCK is suggested to associate with comorbid alcohol use and depressive disorders. *J Circadian Rhythms* 2010; **8**: 1.
69. Bunney Jr WE, Murphy DL, Goodwin FK, Borge GF. The “switch process” in manic-depressive illness. I. A systematic study of sequential behavioral changes. *Arch Gen Psychiatry* 1972; **27**: 295–302.
70. Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. *Addiction* 2006; **101**(Suppl 1): 23–30.
71. Niculescu III AB. Polypharmacy in oligopopulations: what psychiatric genetics can teach biological psychiatry. *Psychiatr Genet* 2006; **16**: 241–244.
72. Sommer W, Arlinde C, Heilig M. The search for candidate genes of alcoholism: evidence from expression profiling studies. *Addict Biol* 2005; **10**: 71–79.
73. Sugawara H, Iwamoto K, Bundo M, Ishiwata M, Ueda J, Kakiuchi C et al. Effect of mood stabilizers on gene expression in lymphoblastoid cells. *J Neural Transm* 2010; **117**: 155–164.
74. Lewis CM, Ng MY, Butler AW, Cohen-Woods S, Uher R, Piro K et al. Genome-wide association study of major recurrent depression in the U.K. population. *Am J Psychiatry* 2010; **167**: 949–957.
75. Szczepankiewicz A, Skibinska M, Hauser J, Słopien A, Leszczynska-Rodziewicz A, Kapelski P et al. Association analysis of the GSK3beta T-50C gene polymorphism with schizophrenia and bipolar disorder. *Neuropsychobiology* 2006; **53**: 51–56.
76. Lachman HM, Pedrosa E, Petruolo OA, Cockerham M, Papolos A, Novak T et al. Increase in GSK3beta gene copy number variation in bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet* 2007; **144B**: 259–265.
77. Kalscheuer VM, Musante L, Fang C, Hoffmann K, Fuchs C, Carta E et al. A balanced chromosomal translocation disrupting ARHGEF9 is associated with epilepsy, anxiety, aggression, and mental retardation. *Hum Mutat* 2009; **30**: 61–68.
78. Kaabi B, Gelemler J, Woods SW, Goddard A, Page GP, Elston RC. Genome scan for loci predisposing to anxiety disorders using a novel multivariate approach: strong evidence for a chromosome 4 risk locus. *Am J Hum Genet* 2006; **78**: 543–553.
79. Gaiteri C, Guilloux JP, Lewis DA, Sibille E. Altered gene synchrony suggests a combined hormone-mediated dysregulated state in major depression. *PLoS One* 2010; **5**: e9970.
80. Thibault C, Lai C, Wilke N, Duong B, Olive MF, Rahman S et al. Expression profiling of neural cells reveals specific patterns of ethanol-responsive gene expression. *Mol Pharmacol* 2000; **58**: 1593–1600.
81. Cichon S, Schumacher J, Müller DJ, Hurter M, Windemuth C, Strauch K et al. A genome screen for genes predisposing to bipolar affective disorder detects a new susceptibility locus on 8q. *Hum Mol Genet* 2001; **10**: 2933–2944.
82. Del Zompo M, Severino G, Ardaur R, Chillotti C, Piccardi M, Dib C et al. Genome-scan for bipolar disorder with sib-pair families in the Sardinian population: a new susceptibility locus on chromosome 1p22-p21? *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 1200–1208.
83. Edenberg HJ, Koller DL, Xuei X, Wetherill L, McClintick JN, Almasy L et al. Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcohol Clin Exp Res* 2010; **34**: 840–852.
84. Bell RL, Kimpel MW, McClintick JN, Strother WN, Carr LG, Liang T et al. Gene expression changes in the nucleus accumbens of alcohol-preferring rats following chronic ethanol consumption. *Pharmacol Biochem Behav* 2009; **94**: 131–147.
85. Sokolov BP, Jiang L, Trivedi NS, Aston C. Transcription profiling reveals mitochondrial, ubiquitin and signaling systems abnormalities in postmortem brains from subjects with a history of alcohol abuse or dependence. *J Neurosci Res* 2003; **72**: 756–767.
86. Etain B, Mathieu F, Rietschel M, Maier W, Albus M, McKeon P et al. Genome-wide scan for genes involved in bipolar affective disorder in 70 European families ascertained through a bipolar type I early-onset proband: supportive evidence for linkage at 3p14. *Mol Psychiatry* 2006; **11**: 685–694.
87. Segman RH, Goltser-Dubner T, Weiner I, Canetti L, Galili-Weisstub E, Milwidsky A et al. Blood mononuclear cell gene expression signature of postpartum depression. *Mol Psychiatry* 2010; **15**: 93–100, 2.
88. Sklar P, Pato MT, Kirby A, Petyshen TL, Medeiros H, Carvalho C et al. Genome-wide scan in Portuguese Island families identifies 5q31-5q35 as a susceptibility locus for schizophrenia and psychosis. *Mol Psychiatry* 2004; **9**: 213–218.
89. Hong KS, McInnes LA, Service SK, Song T, Lucas J, Silva S et al. Genetic mapping using haplotype and model-free linkage analysis supports previous evidence for a locus predisposing to severe bipolar disorder at 5q31-33. *Am J Med Genet B Neuropsychiatr Genet* 2004; **125B**: 83–86.
90. Crowe RR, Goedken R, Samuelson S, Wilson R, Nelson J, Noyes Jr R. Genomewide survey of panic disorder. *Am J Med Genet* 2001; **105**: 105–109.
91. Mukherjee S, Coque L, Cao JL, Kumar J, Chakravarty S, Asaithamb A et al. Knockdown of clock in the ventral tegmental area through RNA interference results in a mixed state of mania and depression-like behavior. *Biol Psychiatry* 2010; **68**: 503–511.
92. Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry* 2005; **57**: 252–260.
93. Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Arch Gen Psychiatry* 1991; **48**: 648–654.
94. Kraschewski A, Reese J, Angheluescu I, Winterer G, Schmidt LG, Gallinat J et al. Association of the dopamine D2 receptor gene with alcohol dependence: haplotypes and subgroups of alcoholics as key factors for understanding receptor function. *Pharmacogenet Genomics* 2009; **19**: 513–527.
95. Munafo MR, Matheson IJ, Flint J. Association of the DRD2 gene Taq1A polymorphism and alcoholism: a meta-analysis of case-control studies and evidence of publication bias. *Mol Psychiatry* 2007; **12**: 454–461.
96. Smith L, Watson M, Gates S, Ball D, Foxcroft D. Meta-analysis of the association of the Taq1A polymorphism with the risk of alcohol dependency: a HuGE gene-disease association review. *Am J Epidemiol* 2008; **167**: 125–138.
97. Ponce G, Perez-Gonzalez R, Aragues M, Palomo T, Rodríguez-Jimenez R, Jimenez-Arriero MA et al. The ANKK1 kinase gene and psychiatric disorders. *Neurotox Res* 2009; **16**: 50–59.
98. Yang BZ, Kranzler HR, Zhao H, Gruen JR, Luo X, Gelemler J. Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. *Alcohol Clin Exp Res* 2008; **32**: 2117–2127.
99. Sipilä T, Kananen L, Greco D, Donner J, Silander K, Terwilliger JD et al. An association analysis of circadian genes in anxiety disorders. *Biol Psychiatry* 2010; **67**: 1163–1170.
100. Massat I, Souery D, Del-Favero J, Van Gestel S, Serretti A, Macciardi F et al. Positive association of dopamine D2 receptor polymorphism with bipolar affective disorder in a European Multicenter Association Study of affective disorders. *Am J Med Genet* 2002; **114**: 177–185.
101. Maron E, Nikopentis T, Koks S, Altmäe S, Heinaste E, Vabrit K et al. Association study of 90 candidate gene polymorphisms in panic disorder. *Psychiatr Genet* 2005; **15**: 17–24.
102. Surget A, Wang Y, Leman S, Ibarguen-Vargas Y, Edgar N, Griebel G et al. Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. *Neuropsychopharmacology* 2009; **34**: 1363–1380.
103. Iwamoto K, Bundo M, Yamamoto M, Ozawa H, Saito T, Kato T. Decreased expression of NEFH and PCP4/PEP19 in the prefrontal cortex of alcoholics. *Neurosci Res* 2004; **49**: 379–385.
104. Miller GE, Chen E, Sze J, Marin T, Arevalo JM, Doll R et al. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. *Biol Psychiatry* 2008; **64**: 266–272.
105. Doyle AE, Biederman J, Ferreira MA, Wong P, Smoller JW, Faraone SV. Suggestive linkage of the child behavior checklist juvenile bipolar disorder phenotype to 1p21, 6p21, and 8q21. *J Am Acad Child Adolesc Psychiatry* 2010; **49**: 378–387.
106. Nash MW, Huez-Diaz P, Williamson RJ, Sterne A, Purcell S, Hoda F et al. Genome-wide linkage analysis of a composite index of neuroticism and mood-related scales in extreme selected sibships. *Hum Mol Genet* 2004; **13**: 2173–2182.
107. Ishikawa M, Mizukami K, Iwakiri M, Asada T. Immunohistochemical and immunoblot analysis of Dopamine and cyclic AMP-regulated phosphoprotein, relative molecular mass 32,000 (DARPP-32) in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; **31**: 1177–1181.
108. Torres KC, Souza BR, Miranda DM, Nicolato R, Neves FS, Barros AG et al. The leukocytes expressing DARPP-32 are reduced in patients with schizophrenia and bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 214–219.
109. Tabakoff B, Saba L, Printz M, Flodman P, Hodgkinson C, Goldman D et al. Genetical genomic determinants of alcohol consumption in rats and humans. *BMC Biol* 2009; **7**: 70.
110. Benes FM, Matzilevich D, Burke RE, Walsh J. The expression of proapoptosis genes is increased in bipolar disorder, but not in schizophrenia. *Mol Psychiatry* 2006; **11**: 241–251.
111. Kawai T, Morita K, Masuda K, Nishida K, Shikishima M, Ohta M et al. Gene expression signature in peripheral blood cells from medical students exposed to chronic psychological stress. *Biol Psychol* 2007; **76**: 147–155.
112. Fallin MD, Lasseter VK, Avramopoulos D, Nicodemus KK, Wolyniec PS, McGrath JA et al. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. *Am J Hum Genet* 2005; **77**: 918–936.
113. McQuillin A, Rizig M, Gurling HM. A microarray gene expression study of the molecular pharmacology of lithium carbonate on mouse brain mRNA to understand the neurobiology of mood stabilization and treatment of bipolar affective disorder. *Pharmacogenet Genomics* 2007; **17**: 605–617.
114. Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA. Gene expression in human alcoholism: microarray analysis of frontal cortex. *Alcohol Clin Exp Res* 2000; **24**: 1873–1882.
115. Cordeiro Q, Talkowski ME, Chowdari KV, Wood J, Nimgaonkar V, Vallada H. Association and linkage analysis of RGS4 polymorphisms with schizophrenia and bipolar disorder in Brazil. *Genes Brain Behav* 2005; **4**: 45–50.
116. Bertsch B, Ogdan CA, Sidhu K, Le-Niculescu H, Kuczenski R, Niculescu AB. Convergent functional genomics: a Bayesian candidate gene identification approach for complex disorders. *Methods* 2005; **37**: 274–279.
117. Aston C, Jiang L, Sokolov BP. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Mol Psychiatry* 2005; **10**: 309–322.

118. Middleton FA, Pato CN, Gentile KL, McGann L, Brown AM, Trauzzi M *et al*. Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches. *Am J Med Genet B Neuropsychiatr Genet* 2005; **136**: 12–25.
119. Zubenko GS, Maher B, Hughes III HB, Zubenko WN, Stiffler JS, Kaplan BB *et al*. Genome-wide linkage survey for genetic loci that influence the development of depressive disorders in families with recurrent, early-onset, major depression. *Am J Med Genet B Neuropsychiatr Genet* 2003; **123**: 1–18.
120. Cassidy F, Zhao C, Badger J, Claffey E, Dobrin S, Roche S *et al*. Genome-wide scan of bipolar disorder and investigation of population stratification effects on linkage: support for susceptibility loci at 4q21, 7q36, 9p21, 12q24, 14q24, and 16p13. *Am J Med Genet B Neuropsychiatr Genet* 2007; **144B**: 791–801.
121. Goes FS, Zandi PP, Miao K, McMahon FJ, Steele J, Willour VL *et al*. Mood-incongruent psychotic features in bipolar disorder: familial aggregation and suggestive linkage to 2p11-q14 and 13q21-33. *Am J Psychiatry* 2007; **164**: 236–247.
122. Rogers PJ, Hohoff C, Heatherley SV, Mullings EL, Maxfield PJ, Evershed RP *et al*. Association of the anxiogenic and alerting effects of caffeine with ADORA2A and ADORA1 polymorphisms and habitual level of caffeine consumption. *Neuropsychopharmacology* 2010; **35**: 1973–1983.
123. Maron E, Kallassalu K, Tammiste A, Kolde R, Vilo J, Toru I *et al*. Peripheral gene expression profiling of CCK-4-induced panic in healthy subjects. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 269–274.
124. Deckert J, Nothen MM, Franke P, Delmo C, Fritze J, Knapp M *et al*. Systematic mutation screening and association study of the A1 and A2a adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. *Mol Psychiatry* 1998; **3**: 81–85.
125. Hamilton SP, Slager SL, De Leon AB, Heiman GA, Klein DF, Hodge SE *et al*. Evidence for genetic linkage between a polymorphism in the adenosine 2A receptor and panic disorder. *Neuropsychopharmacology* 2004; **29**: 558–565.
126. Barley K, Dracheva S, Byne W. Subcortical oligodendrocyte- and astrocyte-associated gene expression in subjects with schizophrenia, major depression and bipolar disorder. *Schizophr Res* 2009; **112**: 54–64.
127. Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G *et al*. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci USA* 1999; **96**: 5604–5609.
128. Liang KY, Wang Y, Shugart YY, Grados M, Fyer AJ, Rauch S *et al*. Evidence for potential relationship between SLC1A1 and a putative genetic linkage region on chromosome 14q to obsessive-compulsive disorder with compulsive hoarding. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 1000–1002.
129. Gelernter J, Page GP, Bonvicini K, Woods SW, Pauls DL, Kruger S. A chromosome 14 risk locus for simple phobia: results from a genomewide linkage scan. *Mol Psychiatry* 2003; **8**: 71–82.
130. Pennington K, Beasley CL, Dicker P, Fagan A, English J, Pariante CM *et al*. Prominent synaptic and metabolic abnormalities revealed by proteomic analysis of the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder. *Mol Psychiatry* 2008; **13**: 1102–1117.
131. Seelan RS, Khalyfa A, Lakshmanan J, Casanova MF, Parthasarathy RN. Deciphering the lithium transcriptome: microarray profiling of lithium-modulated gene expression in human neuronal cells. *Neuroscience* 2008; **151**: 1184–1197.
132. Fullerton JM, Donald JA, Mitchell PB, Schofield PR. Two-dimensional genome scan identifies multiple genetic interactions in bipolar affective disorder. *Biol Psychiatry* 2010; **67**: 478–486.
133. Lambert D, Middle F, Hamshere ML, Segurado R, Raybould R, Corvin A *et al*. Stage 2 of the Wellcome Trust UK-Irish bipolar affective disorder sibling-pair genome screen: evidence for linkage on chromosomes 6q16-q21, 4q12-q21, 9p21, 10p14-p12 and 18q22. *Mol Psychiatry* 2005; **10**: 831–841.
134. Foroud T, Bice P, Castelluccio P, Bo R, Miller L, Ritchoffe A *et al*. Identification of quantitative trait loci influencing alcohol consumption in the high alcohol drinking and low alcohol drinking rat lines. *Behav Genet* 2000; **30**: 131–140.
135. Conti B, Maier R, Barr AM, Morale MC, Lu X, Sanna PP *et al*. Region-specific transcriptional changes following the three antidepressant treatments electro convulsive therapy, sleep deprivation and fluoxetine. *Mol Psychiatry* 2007; **12**: 167–189.
136. Liu J, Lewohl JM, Harris RA, Iyer VR, Dodd PR, Randall PK *et al*. Patterns of gene expression in the frontal cortex discriminate alcoholic from nonalcoholic individuals. *Neuropsychopharmacology* 2006; **31**: 1574–1582.
137. Bergen AW, Yang XR, Bai Y, Beerman MB, Goldstein AM, Goldin LR. Genomic regions linked to alcohol consumption in the Framingham Heart Study. *BMC Genet* 2003; **4** (Suppl 1): S101.
138. Thorgeirsson TE, Oskarsson H, Desnica N, Kostic JP, Stefansson JG, Kolbeinsson H *et al*. Anxiety with panic disorder linked to chromosome 9q in Iceland. *Am J Hum Genet* 2003; **72**: 1221–1230.
139. Wang SS, Kamphuis W, Huitinga I, Zhou JN, Swaab DF. Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. *Mol Psychiatry* 2008; **13**: 786–799, 741.
140. Treutlein J, Cichon S, Ridinger M, Wodarz N, Soyka M, Zill P *et al*. Genome-wide association study of alcohol dependence. *Arch Gen Psychiatry* 2009; **66**: 773–784.
141. Mill J, Kiss E, Baji I, Kapornai K, Daroczy G, Vetro A *et al*. Association study of the estrogen receptor alpha gene (ESR1) and childhood-onset mood disorders. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 1323–1326.
142. Wang H, Zhu YZ, Wong PT, Farook JM, Teo AL, Lee LK *et al*. cDNA microarray analysis of gene expression in anxious PVG and SD rats after cat-freezing test. *Exp Brain Res* 2003; **149**: 413–421.
143. Klempan TA, Rujescu D, Merette C, Himmelman C, Sequeira A, Canetti L *et al*. Profiling brain expression of the spermidine/spermine N1-acetyltransferase 1 (SAT1) gene in suicide. *Am J Med Genet B Neuropsychiatr Genet* 2009; **150B**: 934–943.
144. Maziade M, Roy MA, Chagnon YC, Cliche D, Fournier JP, Montgrain N *et al*. Shared and specific susceptibility loci for schizophrenia and bipolar disorder: a dense genome scan in Eastern Quebec families. *Mol Psychiatry* 2005; **10**: 486–499.
145. Erhardt A, Czibere L, Roeske D, Lucae S, Unschuld PG, Ripke S *et al*. TMEM132D, a new candidate for anxiety phenotypes: evidence from human and mouse studies. *Mol Psychiatry*; advance online publication, 6 April 2010 [e-pub ahead of print].
146. Bilkei-Gorzo A, Racz I, Michel K, Zimmer A, Klingmuller D, Zimmer A. Behavioral phenotype of pre-proenkephalin-deficient mice on diverse congenic backgrounds. *Psychopharmacology (Berl)* 2004; **176**: 343–352.
147. Palo OM, Soronen P, Silander K, Varilo T, Tuononen K, Kieseppa T *et al*. Identification of susceptibility loci at 7q31 and 9p13 for bipolar disorder in an isolated population. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 723–735.
148. Kim KS, Han PL. Optimization of chronic stress paradigms using anxiety- and depression-like behavioral parameters. *J Neurosci Res* 2006; **83**: 497–507.
149. Sherva R, Rice JP, Neuman RJ, Rochberg N, Bierer LJ. Associations and interactions between SNPs in the alcohol metabolizing genes and alcoholism phenotypes in European Americans. *Alcohol Clin Exp Res* 2009; **33**: 848–857.
150. Sequeira A, Klempan T, Canetti L, French-Mullen J, Benkelfat C, Rouleau GA *et al*. Patterns of gene expression in the limbic system of suicides with and without major depression. *Mol Psychiatry* 2007; **12**: 640–655.
151. Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM *et al*. Novel loci for major depression identified by genome-wide association study of Sequenced Treatment Alternatives to Relieve Depression and meta-analysis of three studies. *Mol Psychiatry* 2011; **16**: 202–215.
152. Sherrin T, Blank T, Saravana R, Rayner M, Spiess J, Todorovic C. Region specific gene expression profile in mouse brain after chronic corticotropin releasing factor receptor 1 activation: the novel role for diazepam binding inhibitor in contextual fear conditioning. *Neuroscience* 2009; **162**: 14–22.
153. Fallin MD, Lasseter VK, Wolyniec PS, McGrath JA, Nestadt G, Valle D *et al*. Genomewide linkage scan for bipolar-disorder susceptibility loci among Ashkenazi Jewish families. *Am J Hum Genet* 2004; **75**: 204–219.
154. McBride WJ, Kimpel MW, Schultz JA, McClintock JN, Edenberg HJ, Bell RL. Changes in gene expression in regions of the extended amygdala of alcohol-preferring rats after binge-like alcohol drinking. *Alcohol* 2010; **44**: 171–183.
155. Park N, Juo SH, Cheng R, Liu J, Loth JE, Lilliston B *et al*. Linkage analysis of psychosis in bipolar pedigrees suggests novel putative loci for bipolar disorder and shared susceptibility with schizophrenia. *Mol Psychiatry* 2004; **9**: 1091–1099.
156. Schulze TG, Buenvenich S, Badner JA, Steele CJ, Detera-Wadleigh SD, Dick D *et al*. Loci on chromosomes 6q and 6p interact to increase susceptibility to bipolar affective disorder in the national institute of mental health genetics initiative pedigrees. *Biol Psychiatry* 2004; **56**: 18–23.
157. Sun F, Cheng R, Flanders WD, Yang Q, Khoury MJ. Whole genome association studies for genes affecting alcohol dependence. *Genet Epidemiol* 1999; **17**(Suppl 1): S337–S342.
158. Liebl C, Panhuysen M, Putz B, Trumbach D, Wurst W, Deussing JM *et al*. Gene expression profiling following maternal deprivation: involvement of the brain Renin-Angiotensin system. *Front Mol Neurosci* 2009; **2**: 1.
159. Beasley CL, Pennington K, Behan A, Wait R, Dunn MJ, Cotter D. Proteomic analysis of the anterior cingulate cortex in the major psychiatric disorders: evidence for disease-associated changes. *Proteomics* 2006; **6**: 3414–3425.
160. Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD *et al*. Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. *Mol Psychiatry*; advance online publication, 13 April 2010 [e-pub ahead of print].
161. Dick DM, Nurnberger Jr J, Edenberg HJ, Goate A, Crowe R, Rice J *et al*. Suggestive linkage on chromosome 1 for a quantitative alcohol-related phenotype. *Alcohol Clin Exp Res* 2002; **26**: 1453–1460.
162. Mozhui K, Karlsson RM, Kash TL, Ihne J, Norcross M, Patel S *et al*. Strain differences in stress responsivity are associated with divergent amygdala gene expression and glutamate-mediated neuronal excitability. *J Neurosci* 2010; **30**: 5357–5367.
163. Orsetti M, Di Brisco F, Rinaldi M, Dallorto D, Ghi P. Some molecular effectors of antidepressant action of quetiapine revealed by DNA microarray in the frontal cortex of anhedonic rats. *Pharmacogenet Genomics* 2009; **19**: 600–612.
164. Ising M, Lucae S, Binder EB, Bettecken T, Uhr M, Ripke S *et al*. A genomewide association study points to multiple loci that predict antidepressant drug treatment outcome in depression. *Arch Gen Psychiatry* 2009; **66**: 966–975.

165. Brandish PE, Su M, Holder DJ, Hodor P, Szumiloski J, Kleinhanz RR et al. Regulation of gene expression by lithium and depletion of inositol in slices of adult rat cortex. *Neuron* 2005; **45**: 861–872.
166. Hill SY, Shen S, Zezza N, Hoffman EK, Perlin M, Allan W. A genome wide search for alcoholism susceptibility genes. *Am J Med Genet B Neuropsychiatr Genet* 2004; **128**: 102–113.
167. Pato CN, Pato MT, Kirby A, Petyshen TL, Medeiros H, Carvalho C et al. Genome-wide scan in Portuguese Island families implicates multiple loci in bipolar disorder: fine mapping adds support on chromosomes 6 and 11. *Am J Med Genet B Neuropsychiatr Genet* 2004; **127B**: 30–34.
168. Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP et al. Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc Natl Acad Sci USA* 2005; **102**: 15653–15658.
169. Mitsuyama H, Little KY, Sieghart W, Devaud LL, Morrow AL. GABA(A) receptor alpha1, alpha4, and beta3 subunit mRNA and protein expression in the frontal cortex of human alcoholics. *Alcohol Clin Exp Res* 1998; **22**: 815–822.
170. Song J, Koller DL, Foroud T, Carr K, Zhao J, Rice J et al. Association of GABA(A) receptors and alcohol dependence and the effects of genetic imprinting. *Am J Med Genet B Neuropsychiatr Genet* 2003; **117**: 39–45.
171. Craddock N, Jones L, Jones IR, Kirov G, Green EK, Grozeva D et al. Strong genetic evidence for a selective influence of GABA(A) receptors on a component of the bipolar disorder phenotype. *Mol Psychiatry* 2010; **15**: 146–153.
172. Lind PA, Macgregor S, Vink JM, Pergadia ML, Hansell NK, de Moor MH et al. A genomewide association study of nicotine and alcohol dependence in Australian and Dutch populations. *Twin Res Hum Genet* 2010; **13**: 10–29.
173. O'Dushlaine C, Kenny E, Heron E, Donohoe G, Gill M, Morris D et al. Molecular pathways involved in neuronal cell adhesion and membrane scaffolding contribute to schizophrenia and bipolar disorder susceptibility. *Mol Psychiatry* 2011; **16**: 286–292.
174. Smoller JW, Acierno Jr JS, Rosenbaum JF, Biederman J, Pollack MH, Meminger S et al. Targeted genome screen of panic disorder and anxiety disorder proneness using homology to murine QTL regions. *Am J Med Genet* 2001; **105**: 195–206.
175. Meira-Lima IV, Pereira AC, Mota GF, Krieger JE, Vallada H. Angiotensinogen and angiotensin converting enzyme gene polymorphisms and the risk of bipolar affective disorder in humans. *Neurosci Lett* 2000; **293**: 103–106.
176. Su YA, Wu J, Zhang L, Zhang Q, Su DM, He P et al. Dysregulated mitochondrial genes and networks with drug targets in postmortem brain of patients with posttraumatic stress disorder (PTSD) revealed by human mitochondria-focused cDNA microarrays. *Int J Biol Sci* 2008; **4**: 223–235.
177. Xuei X, Flury-Wetherill L, Almsay L, Bierut L, Tischfield J, Schuckit M et al. Association analysis of genes encoding the nociceptin receptor (OPRL1) and its endogenous ligand (PNOC) with alcohol or illicit drug dependence. *Addict Biol* 2008; **13**: 80–87.
178. Kohen R, Kirov S, Navaja GP, Happe HK, Hamblin MW, Snoddy JR et al. Gene expression profiling in the hippocampus of learned helpless and nonhelpless rats. *Pharmacogenomics J* 2005; **5**: 278–291.
179. Worst TJ, Tan JC, Robertson DJ, Freeman WM, Hyytia P, Kianmaa K et al. Transcriptome analysis of frontal cortex in alcohol-preferring and nonpreferring rats. *J Neurosci Res* 2005; **80**: 529–538.
180. Flatscher-Bader T, van der Brug M, Hwang JW, Goehoe PA, Matsumoto I, Niwa S et al. Alcohol-responsive genes in the frontal cortex and nucleus accumbens of human alcoholics. *J Neurochem* 2005; **93**: 359–370.
181. Ryan MM, Lockstone HE, Huffaker SJ, Wayland MT, Webster MJ, Bahn S. Gene expression analysis of bipolar disorder reveals downregulation of the ubiquitin cycle and alterations in synaptic genes. *Mol Psychiatry* 2006; **11**: 965–978.
182. Morissette J, Villeneuve A, Bordeleau L, Rochette D, Laberge C, Gagne B et al. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in quebec points to a locus of major effect on chromosome 12q23-q24. *Am J Med Genet* 1999; **88**: 567–587.
183. Weissman MM, Fyer AJ, Haghghi F, Heiman G, Deng Z, Hen R et al. Potential panic disorder syndrome: clinical and genetic linkage evidence. *Am J Med Genet* 2000; **96**: 24–35.
184. Soria V, Martinez-Amoros E, Escaramis G, Valero J, Perez-Egea R, Garcia C et al. Differential association of circadian genes with mood disorders: CRY1 and NPAS2 are associated with unipolar major depression and CLOCK and VIP with bipolar disorder. *Neuropsychopharmacology* 2010; **35**: 1279–1289.
185. Maron E, Hetta JM, Shlik J. Advances in molecular genetics of panic disorder. *Mol Psychiatry* 2010; **15**: 681–701.
186. Luciano M, Houlihan LM, Harris SE, Gow AJ, Hayward C, Starr JM et al. Association of existing and new candidate genes for anxiety, depression and personality traits in older people. *Behav Genet* 2010; **40**: 518–532.
187. Hamilton SP, Fyer AJ, Durner M, Heiman GA, Baisre de Leon A, Hodge SE et al. Further genetic evidence for a panic disorder syndrome mapping to chromosome 13q. *Proc Natl Acad Sci USA* 2003; **100**: 2550–2555.
188. Fyer AJ, Hamilton SP, Durner M, Haghghi F, Heiman GA, Costa R et al. A third-pass genome scan in a panic disorder: evidence for multiple susceptibility loci. *Biol Psychiatry* 2006; **60**: 388–401.
189. Ishikawa M, Mizukami K, Iwakiri M, Hidaka S, Asada T. Immunohistochemical and immunoblot study of GABA(A) alpha1 and beta2/3 subunits in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci Res* 2004; **50**: 77–84.
190. Johnson C, Drgon T, McMahon FJ, Uhl GR. Convergent genome wide association results for bipolar disorder and substance dependence. *Am J Med Genet B Neuropsychiatr Genet* 2009; **150B**: 182–190.
191. Xing G, Zhang L, Russell S, Post R. Reduction of dopamine-related transcription factors Nurr1 and NGFI-B in the prefrontal cortex in schizophrenia and bipolar disorders. *Schizophr Res* 2006; **84**: 36–56.
192. Etheridge N, Lewohl JM, Mayfield RD, Harris RA, Dodd PR. Synaptic proteome changes in the superior frontal gyrus and occipital cortex of the alcoholic brain. *Proteomics Clin Appl* 2009; **3**: 730–742.
193. Dick DM, Aliev F, Bierut L, Goate A, Rice J, Hinrichs A et al. Linkage analyses of IQ in the collaborative study on the genetics of alcoholism (COGA) sample. *Behav Genet* 2006; **36**: 77–86.
194. Benes FM, Todtenkopf MS, Logiotatos P, Williams M. Glutamate decarboxylase(65)-immunoreactive terminals in cingulate and prefrontal cortices of schizophrenic and bipolar brain. *J Chem Neuroanat* 2000; **20**: 259–269.
195. Heckers S, Stone D, Walsh J, Shick J, Koul P, Benes FM. Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. *Arch Gen Psychiatry* 2002; **59**: 521–529.
196. Lappalainen J, Krupitsky E, Kranzler HR, Luo X, Remizov M, Pchelina S et al. Mutation screen of the GAD2 gene and association study of alcoholism in three populations. *Am J Med Genet B Neuropsychiatr Genet* 2007; **144B**: 183–192.
197. Unschuld PG, Ising M, Specht M, Erhardt A, Ripke S, Heck A et al. Polymorphisms in the GAD2 gene-region are associated with susceptibility for unipolar depression and with a risk factor for anxiety disorders. *Am J Med Genet B Neuropsychiatr Genet* 2009; **150B**: 1100–1109.
198. Martin MV, Rollins B, Sequeira PA, Mesen A, Byerley W, Stein R et al. Exon expression in lymphoblastoid cell lines from subjects with schizophrenia before and after glucose deprivation. *BMC Med Genomics* 2009; **2**: 62.
199. Schuckit MA, Edenberg HJ, Kalmijn J, Flury L, Smith TL, Reich T et al. A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res* 2001; **25**: 323–329.
200. Karssen AM, Her S, Li JZ, Patel PD, Meng F, Bunney Jr WE et al. Stress-induced changes in primate prefrontal profiles of gene expression. *Mol Psychiatry* 2007; **12**: 1089–1102.
201. Derijk RH, van Leeuwen N, Klok MD, Zitman FG. Corticosteroid receptor-gene variants: modulators of the stress-response and implications for mental health. *Eur J Pharmacol* 2008; **585**: 492–501.
202. Weaver IC, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci USA* 2006; **103**: 3480–3485.
203. Jurata LW, Bukhman YV, Charles V, Capriglione F, Bullard J, Lemire AL et al. Comparison of microarray-based mRNA profiling technologies for identification of psychiatric disease and drug signatures. *J Neurosci Methods* 2004; **138**: 173–188.
204. Youngs RM, Chu MS, Meloni EG, Naydenov A, Carlezon Jr WA, Konradi C. Lithium administration to preadolescent rats causes long-lasting increases in anxiety-like behavior and has molecular consequences. *J Neurosci* 2006; **26**: 6031–6039.
205. McNamara RK, Ostrander M, Abplanalp W, Richtand NM, Benoit SC, Clegg DJ. Modulation of phosphoinositide-protein kinase C signal transduction by omega-3 fatty acids: implications for the pathophysiology and treatment of recurrent neuropsychiatric illness. *Prostaglandins Leukot Essent Fatty Acids* 2006; **75**: 237–257.
206. Pandey GN, Dwivedi Y, Ren X, Rizavi HS, Roberts RC, Conley RR et al. Altered expression and phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) in postmortem brain of suicide victims with or without depression. *J Psychiatr Res* 2003; **37**: 421–432.
207. Hattori E, Toyota T, Ishitsuka Y, Iwayama Y, Yamada K, Ujike H et al. Preliminary genome-wide association study of bipolar disorder in the Japanese population. *Am J Med Genet B Neuropsychiatr Genet* 2009; **150B**: 1110–1117.
208. Chu TT, Liu Y, Kemether E. Thalamic transcriptome screening in three psychiatric states. *J Hum Genet* 2009; **54**: 665–675.
209. Maron E, Hetta JM, Shlik J. Advances in molecular genetics of panic disorder. *Mol Psychiatry* 2010; **15**: 681–701.
210. Stopkova P, Saito T, Fann CS, Papolos DF, Vevera J, Paclt I et al. Polymorphism screening of PIP5K2A: a candidate gene for chromosome 10p-linked psychiatric disorders. *Am J Med Genet B Neuropsychiatr Genet* 2003; **123B**: 50–58.
211. Wray NR, James MR, Mah SP, Nelson M, Andrews G, Sullivan PF et al. Anxiety and comorbid measures associated with PLXNA2. *Arch Gen Psychiatry* 2007; **64**: 318–326.
212. Curtis D, Kalsi G, Brynjolfsson J, McInnis M, O'Neill J, Smyth C et al. Genome scan of pedigrees multiply affected with bipolar disorder provides further support for the presence of a susceptibility locus on chromosome 12q23-q24, and suggests the presence of additional loci on 1p and 1q. *Psychiatr Genet* 2003; **13**: 77–84.
213. Gelernter J, Bonvicini K, Page G, Woods SW, Goddard AW, Kruger S et al. Linkage genome scan for loci predisposing to panic disorder or agoraphobia. *Am J Med Genet* 2001; **105**: 548–557.
214. Foroud T, Buchholz KK, Edenberg HJ, Goate A, Neuman RJ, Porjesz B et al. Linkage of an alcoholism-related severity phenotype to chromosome 16. *Alcohol Clin Exp Res* 1998; **22**: 2035–2042.

215. Macgregor S, Visscher PM, Knott SA, Thomson P, Porteous DJ, Millar JK *et al*. A genome scan and follow-up study identify a bipolar disorder susceptibility locus on chromosome 1q42. *Mol Psychiatry* 2004; **9**: 1083–1090.
216. Nakatani N, Ohnishi T, Iwamoto K, Watanabe A, Iwayama Y, Yamashita S *et al*. Expression analysis of actin-related genes as an underlying mechanism for mood disorders. *Biochem Biophys Res Commun* 2007; **352**: 780–786.
217. Del Zompo M, Severino G, Ardau R, Chillotti C, Piccardi M, Dib C *et al*. Genome-scan for bipolar disorder with sib-pair families in the Sardinian population: a new susceptibility locus on chromosome 1p22-p21? *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 1200–1208.
218. Nurnberger Jr JI, Foroud T, Flury L, Su J, Meyer ET, Hu K *et al*. Evidence for a locus on chromosome 1 that influences vulnerability to alcoholism and affective disorder. *Am J Psychiatry* 2001; **158**: 718–724.
219. Zandi PP, Badner JA, Steele J, Willour VL, Miao K, MacKinnon DF *et al*. Genome-wide linkage scan of 98 bipolar pedigrees and analysis of clinical covariates. *Mol Psychiatry* 2007; **12**: 630–639.
220. Bailer U, Leisch F, Meszaros K, Lenzinger E, Willinger U, Strobl R *et al*. Genome scan for susceptibility loci for schizophrenia and bipolar disorder. *Biol Psychiatry* 2002; **52**: 40–52.
221. Sousa JC, Grandela C, Fernandez-Ruiz J, de Miguel R, de Sousa L, Magalhaes AI *et al*. Transthyretin is involved in depression-like behaviour and exploratory activity. *J Neurochem* 2004; **88**: 1052–1058.



Translational Psychiatry is an open-access journal published by Nature Publishing Group. This work is licensed under the Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>

Supplementary Information accompanies the paper on the Translational Psychiatry website (<http://www.nature.com/tp>)