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

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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.
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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 erythrocytes[7] 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 alcoholism[20] and schizophrenia,[21] using a convergent functional genomics

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(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 design[23] 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′-GCAAAGCTCCTTTCTTTGCGAGAAGTGC-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 work[22] 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 described[22] 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 cm^3 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 ± 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 biotinlylate 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,

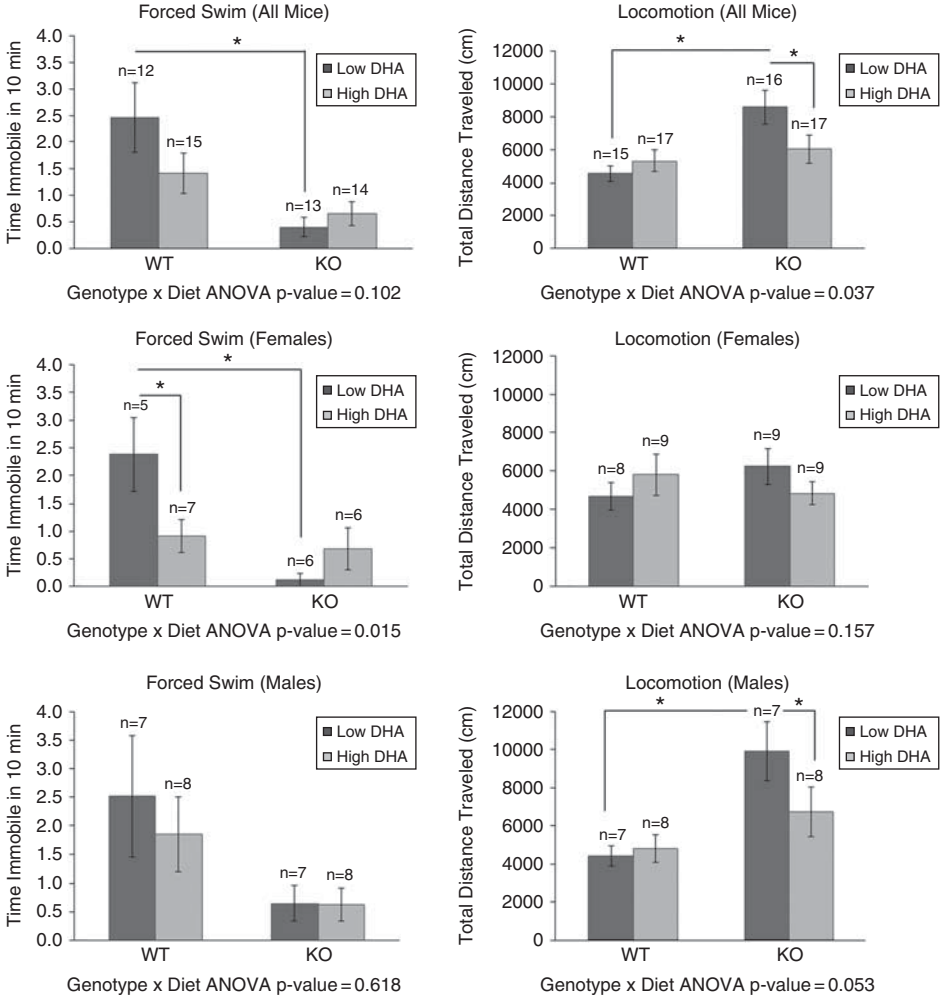

Figure 1 Effects of docosahexaenoic acid (DHA) on stressed mice behavior: DBP (+/+) wild-type (WT) and DBP (−/−) knockout (KO) mice on a diet either high or low in DHA were subjected to a chronic stress paradigm consisting of isolation (single housing) for 28 days, with an acute stressor (behavioral 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 × 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 out[22] (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

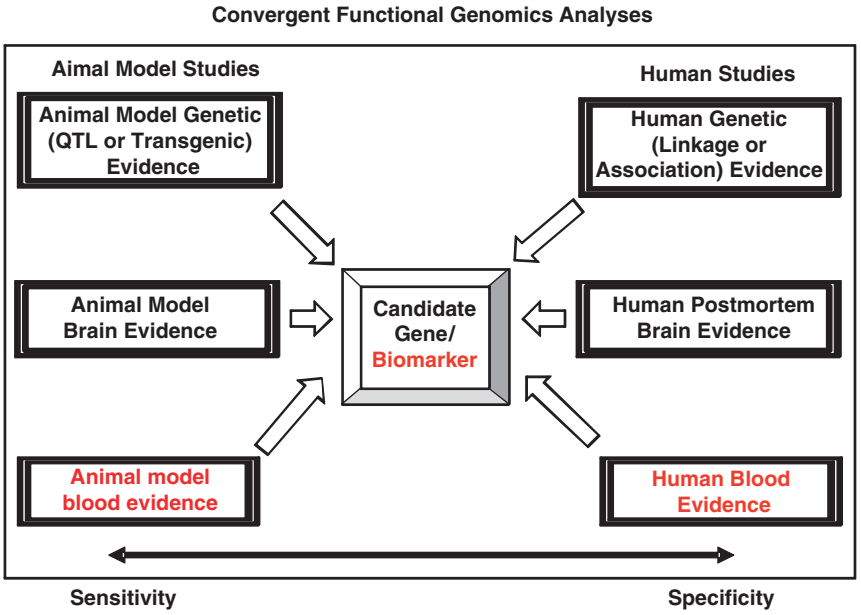

Figure 2 Convergent functional genomics (CFG). Bayesian integration of multiple animal model and human lines of evidence to prioritize disease-relevant genes.

male mice,[22] Resting Time was the phene 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/DARRPP-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 bipolars[40] 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 bipolars[48] as well as in animal models of anxiety[49] 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 bipolars[51] 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 3 β)	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]	3.0
PTTG1 (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*
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 signaling 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
MAL (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
ADORA2A (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
GALC (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] OCD[128] Simple phobia[129]	4.0
PRDX2 (peroxiredoxin 2)	I	(D) DBP-ST PFC[22] Alcohol PFC[116]	BP[30]	(QTL) Abnormal emotion/affect behavior Abnormal circadian rhythm	(D) BP[130] (I) Response to lithium treatment[131]		19p13.2 (linkage) BP[132] MDD[119]	4.0
TAC1 (tachykinin 1)	I	(I) DBP-ST AMY[22]		(Transgenic) Decreased anxiety-related response, increased coping response	(I) MDD[79]		7q21.3 (linkage) BP[133] Alcohol[134]	3.5
NR4A3 (nuclear receptor subfamily 4, group A, member 3)	D	(I) Alcohol[84] (D) MDD-Fluoxetine[135]		(QTL) Addiction/drug abuse	(I) Alcohol[136]	(I) PTSD[43]	9q22.33 (linkage) Alcohol[137] Anxiety, PD[138]	3.5
ESR1 (estrogen receptor 1 α)	D	(I) Stress[50]		(Transgenic) Increased aggression	(I) Alcohol[85] (I) MDD[139]		6q25.1 (association) Alcohol[140] Childhood onset mood disorder[141]	3.5
GABRD (γ-aminobutyric acid (GABA) A receptor, subunit δ)	I	(D) MDD-Fluoxetine[135] (I) Anxiety[142] (D) Alcohol[72] Alcohol CP, HIP[116]		(Transgenic) Decreased anxiety-related response	(I) Suicide-MDD[41,143]		18q12.2 (linkage) BP[144]	3.5
CRIM1 (cysteine rich transmembrane BMP regulator 1)	D	(D) Depression[102] (D) DBP-ST PFC[22]		(QTL) Addiction/drug abuse	(D) Suicide-MDD[41]		2p22.3 (association) PD[145]	3.5
PENK (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
ALDH1A (aldehyde dehydrogenase family 1, subfamily A1)	I	(D) Anxiety/Depression[148] (I) DBP-ST AMY[22]	BP[30]	(QTL) Abnormal circadian rhythm			9q21.13 (association) Alcohol[149]	3.5
CADPS2 (Ca2+-dependent activator protein for secretion 2)	D	Alcohol CP[116]		(Transgenic) Decreased exploration in new environment, abnormal circadian rhythm, abnormal sleep pattern	(I) Suicide-MDD[41]		7q31.32 (linkage) BP[147]	3.5
GFRA2 (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
HPCAL1 (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**
CTNNB1 (catenin (cadherin 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
GRIA2 (glutamate receptor, ionotropic, AMPA2 α 2)	D	(I) Alcohol[154] (I) BP[91] (I) Stress[50] (I) Alcohol[72]	BP[30]	(Transgenic) Abnormal anxiety-related response	(I) BP[40] (I) Suicide-MDD[41]		4q32.1 (association) BP[39]	5.0
GJA1 (gap junction protein, α 1)	D	(I) Alcohol[72] Alcohol, HIP[116]	BP[30]	(Transgenic) Abnormal suckling behavior	(I) Alcohol[103]		6q22.31 (linkage) BP[155,156] Alcohol[157]	4.5
GLUL (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]		1q25.3 (linkage) Alcohol[161]	4.0
HOMER1 (homer homolog 1)	D	(D) MDD-Fluoxetine[135] (D) Anxiety[162] (D) Stress[163] Alcohol HIP[116]		(Transgenic) Abnormal response to addictive substance		(D) PTSD[43]	5q14.1 (association) MDD[164]	4.0
PAM (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
GABRB3 (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[46,171]	3.5
NCALD (neurocalcin δ)	D	BP AMY, CP[29]		(QTL) Addiction/drug abuse	(I) Suicide-MDD[41]		8q22.3 (association) Alcohol[172]	3.5
OGT (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
PTTG1 (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 acumbens; 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 $\geqslant 3.5$ 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*
PTGDS (prostaglandin D2 synthase)	I	(D) Anxiety[49] (D) Stress[50]		(Transgenic) Decreased aggression	(D) Alcohol[114]	(D) BP[48]	9q34.3 (association) Anxiety[47]	5.0
ARF3 (ADP-ribosylation factor 3)	I	(D) DBP-ST PFC[22]		(QTL) Addiction/drug abuse	(D) Alcohol[114]	(I) BP[73] (D) Chronic stress[104]	12q13.12 (linkage) PD[174]	4.0
NFIA (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[81,82]	4.0
KLF4 (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
PTTG1 (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*
AGT (angiotensinogen)	I	BP NAC[29] Alcohol CP, HIP, NAC, PFC[116] (D) DBP-NST AMY[22]			(D) Alcohol[114,136]		1q42.2 (association) BP[175]	4.0
HPCAL1 (hippocalcin-like 1)	I	(D) DBP-ST AMY[22]		(QTL) Abnormal circadian rhythm; addiction/drug abuse	(I) Suicide-MDD[41]		2p25.1 (association) MDD[151]	3.5
PNOC (prepronociceptin)	I	(D) DBP-NST AMY[22]		(QTL) Abnormal sleep pattern/ circadian rhythm	(D) PTSD[176]		8p21.1 (association) Alcohol[177]	3.5
SYT1 (synaptotagmin I)	D	(D) Depression[178] (D) Alcohol[179] BP AMY[29]		(Transgenic) Abnormal suckling behavior	(D) Alcohol[180] (D) BP[181]		12q21.2 (linkage) BP[182] PD[183] Alcohol[137]	3.5
PER3 (period homolog 3)	D	(D) BP[113]		(Transgenic) Shortened circadian period			1p36.23 (association) BP[184] PD[185] Alcohol[170] Depression[186]	2.5
PTTG1 (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*
FOS (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, MDD[36]	(I) Stress[44]	5q35.1 (linkage) PD[187,188]	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]		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]	(I) Mood[30]	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] Alcohol[161]	5.0
NR4A2 (nuclear receptor subfamily 4, group A, member 2)	I	(D) Anxiety[162] (D) Stress[50]		(Transgenic) Abnormal suckling behavior	(D) BP, MDD[191]	(D) Mood[30]	2q24.1 (linkage) BP[81]	4.5
CNP (2′,3′-cyclic nucleotide 3′ phosphodiesteras)	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] Anxiety/affective disorder[197]	4.0
GLS (glutaminase)	I			(QTL) Addiction/drug abuse	(D) BP[198]	(D) PTSD[43]	2q32.2 (linkage) Alcohol[166,199]	4.0
GLUL (glutamate-ammonia ligase)[a]	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] 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) Stress[111]	1p31.3 (linkage) BP[81,82]	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]		Xq21.1	3.5
KCNMA1 (potassium large conductance calcium-activated channel, subfamily M, α member 1)	I			(QTL) Abnormal emotion/affect behavior, addiction/drug abuse	(I) MDD[79]		10q22.3 (association) Alcohol[83]	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*
LPAR1 (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[138] BP[155]	3.5
MAPT (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[86,132]	3.5
MARCKS (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
MBNL1 (muscleblind-like 1)	I		BP[30]	(QTL) Abnormal emotion/affect behavior; Abnormal circadian rhythm	(I) MDD[79]		3q25.1 (association) BP[207]	3.5
NCALD (neurocalcin δ)	I			(QTL) Addiction/drug abuse	(I) Suicide-MDD[41]		8q22.3 (association) Alcohol[172]	3.5
NRXN1 (neurexin I)	I	(D) BP[113]		(QTL) Addiction/drug abuse Abnormal emotion/affect behavior	(D) BP[208] (I) Suicide-MDD[41]		2p16.3 (association) BP[173] PD[209]	3.5
PIP4K2A (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
PLXNA2 (plexin A2)	I			(QTL) Addiction/drug abuse Abnormal emotion/affect behavior	(I) Alcohol[136]	(D) Mood[30]	1q32.2 (association) Anxiety[211] BP[207]	3.5
SERPINI1 (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
SNN (stannin)	D	(D) Primate stress-induced[200]		(QTL) Addiction/drug abuse	(I) Alcohol[136]		16p13.13 (linkage) Alcohol[214] BP[86]	3.5
PTTG1 (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 acumbens; 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.
[a]Blood 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 $\geqslant 3.5$ points). Myelin-related genes are underlined.

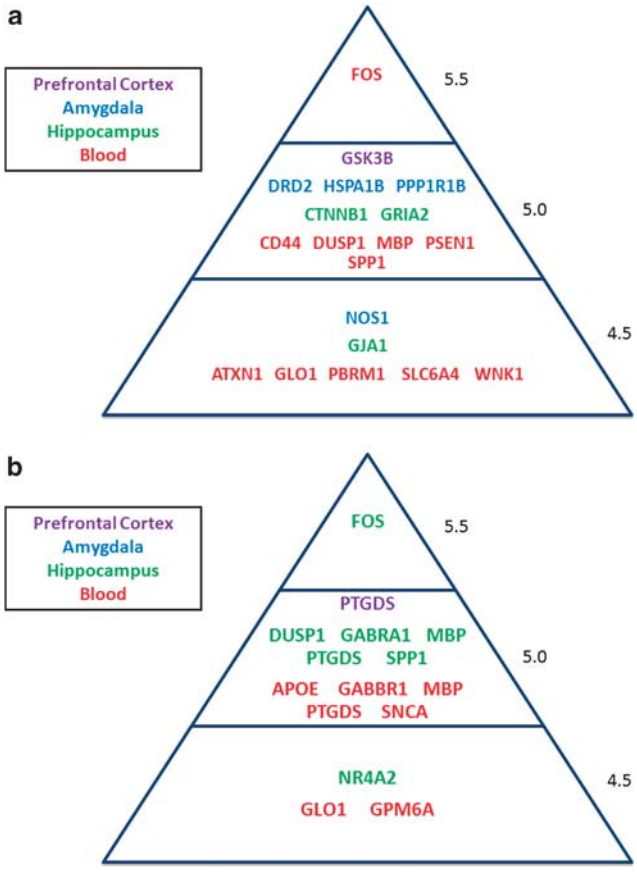

Figure 3 Top candidate genes changed in DBP knockout (KO) stressed (ST) mice on high- vs low-docosahexaenoic acid (DHA) diet. (**a**) Female mice and (**b**) male mice.

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)		
*Top canonical pathways, female PFC (*n = *66 genes)*		
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)
*Top canonical pathways, female AMY (*n = *150 genes)*		
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)
*Top canonical pathways, female HIP (*n = *103 genes)*		
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)		
*Top canonical pathways, male PFC (*n = *77 genes)*		
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)
*Top canonical pathways, male AMY (*n = *59 genes)*		
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)
*Top canonical pathways, male HIP (*n = *352 genes)*		
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, g-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 V*PS13A* (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
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)	I	Alcohol NAC[116]						1.0
VPS13A (vacuolar protein sorting 13A)	D	(I) DBP-ST PFC[22] (I) Depression[102]						1.0
CAPZB (capping protein (actin filament) muscle Z-line, β)	D	(I) Alcohol[154]						0.5
GNG2 (guanine nucleotide binding protein (G protein), γ 2)	D							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***
TFRC (transferrin receptor)	I	Alcohol CP, HIP[116]					3q29 (linkage) BP[219] BP/SZ[220] SZ, SZA, BP[144] Alcohol[161]	1.5
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)	I	(D) DBP-ST AMY[22]	BP[30]					2.0
FTL1 (ferritin light chain 1)	I	(D) DBP-ST AMY; (D) DBP-ST PFC[22] Alcohol NAC[116]						1.0
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-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

Table 4 (Continued)

(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*
LIMD2 (LIM domain containing 2)	D		BP[30]					1.0
TTR (transthyretin)	D	BP CP[29] (I) Anxiety/ depression[148] (I) Anxiety[49,142] (I) Depression[221]						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 acumbens; 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 humans[55] 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			
GSK3B (glycogen synthase kinase 3 β)	Kinase	Enzastaurin	5.0
KCNMA1 (potassium large conductance calcium-activated channel, subfamily M, α member 1)	Ion channel	Tedisamil	3.5
AMY			
DRD2 (dopamine receptor D2)	G-protein-coupled receptor	Paliperidone, risperidone, buspirone, bifeprunox, iloperidone, blonanserin, asenapine, pardoprunox, ocaperidone, abaperidone, SLV-314, RGH-188, rotigotine, opipramol, chloropromazine, metoclopramide, sulpiride, meloxicam, amantadine, trifluoperazine, fluphenazine, pimozide, clozapine, haloperidol, fluoxetine/olanzapine, fluphenazine decanoate, thiothixene, amitriptyline/perphenazine, haloperidol decanoate, molindone, trimethobenzamide	5.0
NOS1 (nitric oxide synthase 1) (neuronal)	Enzyme	GW 273629, omega-N-methylarginine	4.5
ALDH1A1 (aldehyde dehydrogenase 1 family, member A1)	Enzyme	Disulfiram, chlorpropamide	3.5
ESR1 (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
GABRD (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
PDE7B (phosphodiesterase 7B)	Enzyme	Dyphylline, nitroglycerin, aminophylline, anagrelide, milrinone, dipyridamole, tolbutamide, theophylline, pentoxifylline	1.0
SCN4B (sodium channel, voltage-gated, type IV, β)	Ion channel	Riluzole	1.0
HIP			
GRIA2 (glutamate receptor, ionotropic, AMPA 2)	Ion channel	Talampanel, Org 24448, LY451395, tezampanel	5.0
GABRB3 (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
CACNA2D1 (calcium channel, voltage-dependent, α 2/δ subunit 1)	Ion channel	Amlodipine/valsartan/hydrochlorothiazide, amlodipine/telmisartan, bepridil, amlodipine, pregabalin	1.0
IFNGR2 (interferon γ receptor 2 (interferon γ transducer 1))	Transmembrane receptor	Interferon γ-1b	1.0

(b) Males

PFC			
COL6A2 (collagen, type VI, α 2)	Other	Collagenase clostridium histolyticum	1.0
CCR5 (chemokine (C-C motif) receptor 5)	G-protein-coupled receptor	Maraviroc, vicriviroc, SCH 351125	0.5
AMY			
GRIN2C (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*
HIP			
GABRA1 (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
GAD2 (glutamate decarboxylase 2)	Enzyme	Valproic acid	4.0
NR3C2 (nuclear receptor subfamily 3, group C, member 2)	Ligand-dependent nuclear receptor	Hydrochlorothiazide/spironolactone, fludrocortisone acetate, drospirenone, spironolactone, eplerenone	4.0
SLC12A2 (solute carrier family 12 (sodium/potassium/chloride transporters), member 2)	Transporter	Bumetanide	4.0
KCNMA1 potassium large conductance calcium-activated channel, subfamily M, α member 1	Ion channel	Tedisamil	3.5
ATP1A2 (ATPase, Na+/K+ transporting, α 2 polypeptide)	Transporter	Digoxin, omeprazole, ethacrynic acid, perphenazine	2.5
LPL (lipoprotein lipase)	Enzyme	Nicotinic acid, lovastatin/niacin	2.0
SLC1A3 (solute carrier family 1 (glial high affinity glutamate transporter), member 3)	Transporter	Riluzole	2.0
SLC6A1 (solute carrier family 6 (neurotransmitter transporter, GABA), member 1)	Transporter	Tiagabine	2.0
CHUK (conserved helix-loop-helix ubiquitous kinase)	Kinase	Methyl 2-cyano-3,12-dioxoolean-1,9-dien-28-oate	1.0
PARP1 (poly (ADP-ribose) polymerase 1)	Enzyme	ABT-888, INO-1001	1.0
SCN1A (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
TGFB2 (transforming growth factor, β 2)	Growth factor	AP-12009	1.0
HTR5A (5-hydroxytryptamine (serotonin) receptor 5A)	G-protein-coupled receptor	Asenapine	0.5
SCN8A (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 work[22] 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

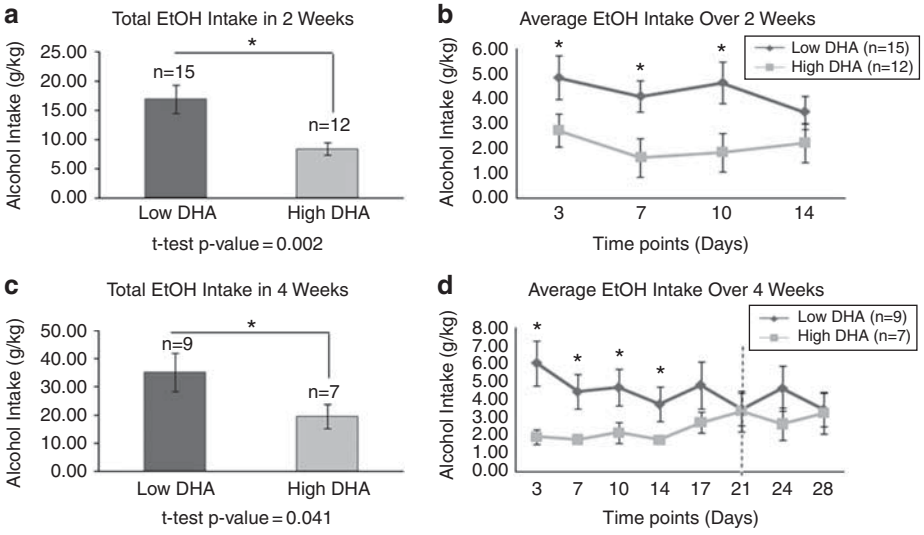

Figure 4 Effects of docosahexaenoic acid (DHA) on male DBP knockout (KO) stressed (ST) mice alcohol (EtOH) consumption: mice on a diet supplemented with either low or high DHA were subjected to alcohol free-choice drinking paradigm. (**a**, **b**) Fluid consumption (water or 10% ethanol) monitored for 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. $*P < 0.05$.

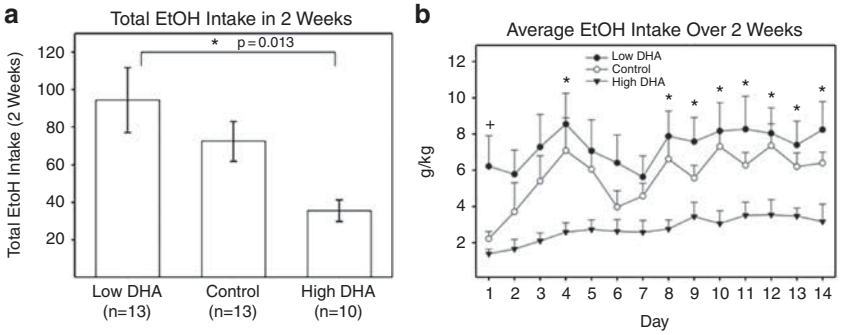

Figure 5 Effects of docosahexaenoic acid (DHA) on alcohol (EtOH) consumption in male alcohol-preferring (P) rats. Experimentally naive, male P rats, 4–6 months of age at the start of the experiment, were used as subjects. These rats were placed on one of the three diets: (1) low-DHA diet, (2) control 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 patients[69] 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, heterogeneity, overlap and interdependence of major psychiatric syndromes as currently defined by DSM[71] 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

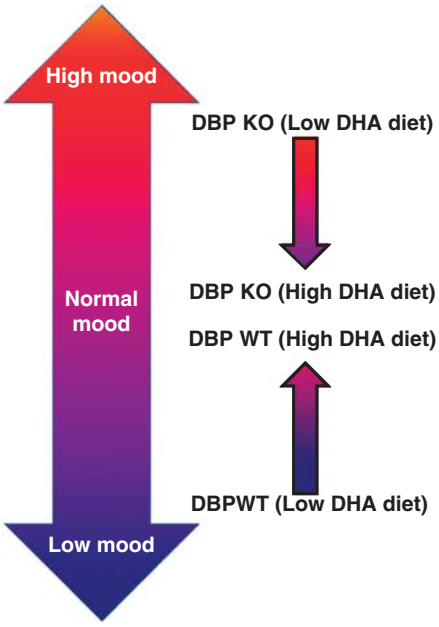

Figure 6 High-docosahexaenoic acid (DHA) diet has a stabilizing effect on mood in stressed mice. A model integrating the behavioral and genomic data.

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.

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