Proteomic and Metabolomic Profiling of a Trait Anxiety Mouse Model Implicate Affected Pathways

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Depression and anxiety disorders affect a great number of people worldwide. Whereas singular factors have been associated with the pathogenesis of psychiatric disorders, growing evidence emphasizes the significance of dysfunctional neural circuits and signaling pathways. Hence, a systems biology approach is required to get a better understanding of psychiatric phenotypes such as depression and anxiety. Furthermore, the availability of biomarkers for these disorders is critical for improved diagnosis and monitoring treatment response. In the present study, a mouse model presenting with robust high versus low anxiety phenotypes was subjected to thorough molecular biomarker and pathway discovery analyses. Reference animals were metabolically labeled with the stable 15N isotope allowing an accurate comparison of protein expression levels between the high anxiety-related behavior versus low anxiety-related behavior mouse lines using quantitative mass spectrometry. Plasma metabolomic analyses identified a number of small molecule biomarkers characteristic for the anxiety phenotype with particular focus on myo-inositol and glutamate as well as the intermediates involved in the tricarboxylic acid cycle. In silico analyses suggested pathways and subnetworks as relevant for the anxiety phenotype. Our data demonstrate that the high anxiety-related behavior and low anxiety-related behavior mouse model is a valuable tool for anxiety disorder drug discovery efforts. Molecular & Cellular Proteomics 10: 10.1074/mcp.M111.008110, 1–11, 2011.

For an improved understanding of the etiology of complex diseases such as psychiatric disorders the elucidation of molecular pathways is critical. In this regard biomarker information can deliver valuable data not only on individual molecular entities but at the same time on pathways critical for disease pathobiology, thus yielding important information for the development of therapeutic agents.

Animal models have the capability to mimic certain aspects of complex disorders and thereby untangle complicated phenotypes such as anxiety, which can be measured in the mouse with the help of the elevated plus maze (EPM) and other anxiety tests (1). In earlier studies we have identified proteome differences in a mouse model of extremes in trait anxiety that are qualitative and quantitative in nature. Whereas the enzyme enolase phosphatase was found as a different isoform in high (HAB) versus low (LAB) anxiety-related behavior mice, another enzyme, glyoxalase-1 (Glx1), showed altered expression levels between the two lines (1, 2). Our approach thus considers the two poles of the continuum “anxiety”: vulnerability of individuals with high risk scores as well as resilience of individuals with low risk scores at the often neglected “other end” of the continuum of polygenic liability (3).

In order to analyze the proteomes of the HAB and LAB mouse lines in greater detail, we have used a comprehensive and accurate proteomics platform that involves metabolic labeling of live animals with stable isotopes followed by quantitative mass spectrometry (4–6). Complementary metabonomic studies provide additional information on pathways affected in disease pathobiology. Here we present results from our proteomic, metabolomic, and pathway analyses of HAB versus LAB mice and discuss their significance with regard to the anxiety phenotype. Our results demonstrate that the mouse model under investigation reflects several critical aspects of human anxiety pathobiology, making it a valuable tool for guiding drug discovery efforts.

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1 The abbreviations used are: EPM, elevated plus maze; HAB, high anxiety-related behavior; LAB, low anxiety-related behavior; TCA, tricarboxylic acid; Glx1, glyoxalase-1; PND, postnatal day; FDR, false discovery rate; PVDF, polyvinylidene fluoride; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CA2, carbonic anhydrase 2; TTR, transthyretin; SAP, serum amyloid P-component.
Male mice from PND56 were used for quantitative proteomics. The total immobility scored by a trained observer blind to the treatment.

Animal Experiments—All the animal experiments were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals of the Government of Bavaria.” The 15N or 14N feeding was started in utero as described previously (5). Feeding the mice with bacterial protein-based diets did not result in any discernible health effects compared with animals fed a standard diet. The mice gained weight similarly to those fed by normal diet. On postnatal day (PND) 56, all male mice were sacrificed and brain sections including the hippocampus were removed for subsequent analyses. Blood was taken by cardiac puncture, and plasma was obtained by centrifuging the blood in an EDTA and protease inhibitor mixture table (F. Hoffmann-La Roche Ltd, Basel, Switzerland) pre-added tube at 1300 × g for 10 min. The pellets representing blood cells were saved. The remaining body blood was removed by 0.9% saline perfusion. Hippocampus and plasma were snap-frozen in liquid nitrogen and stored at −80 °C.

Behavioral Tests—Ultrasonic vocalization tests, EPM, and tail suspension test were performed on PND 5, 49, and 51, respectively, as described previously (5). Briefly, ultrasonic vocalization calls were detected and recorded for 5 min with a bat detector (Mini 3 bat-detector, Ultra Sound Advice, London, U.K.) at 70 kHz. EPM test described previously (5). Briefly, ultrasonic vocalization calls were counted.

Pension test were performed on PND 5, 49, and 51, respectively, as described previously (5). Briefly, ultrasonic vocalization calls were counted.

The hippocampus and plasma of male mice from PND56 were used for quantitative proteomics. The 14N-HAB and 14N-LAB specimens were mixed with the respective 15N-labeled reference material from “normal” anxiety-related behavior (NAB) mice at a 1:1 ratio. HAB/LAB protein expression level differences were deduced by comparing the results from the HAB/NAB and LAB/NAB analyses. This way any potential dietary and/or isotopic effects on protein expression can be avoided a priori. Three biological replicate analyses were conducted for each comparison.

14N-LAB and 15N-HAB plasma samples were mixed with 15N-NAB plasma at a ratio of 1:1. After diluting 1:50 with 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, provided as part of an IgY-M7 Spin column kit (GenWay Biotech, Inc., San Diego, CA), protein concentrations were estimated by Bradford assay. The protein mixtures were then subjected to ultracentrifugation for 1 h at 100,000 × g in a swing bucket rotor at 4 °C. The supernatants were collected as microsome proteins after centrifugation at 9000 × g, 4 °C for 30 min. CytoS and microscopic fraction protein concentrations were measured by Bradford assay.

The brain and plasma protein mixtures were resolved by Criterion XT Bis-Tris precast gels (BioRad), and the gels stained with Coomassie Brilliant Blue (BioRad). The gel lane containing the separated proteins was cut into 2-mm wide pieces, and the resulting pieces subjected to in-gel tryptic digestion. Gel pieces were destained twice with 100 μl 50 mM NH4HCO3/acetonitrile (1:1, v/v) for 30 min, and disulfide bonds reduced with 10 mM dithiotreitol in 50 mM NH4HCO3, pH 8.0 for 30 min, and then alkylated with 55 mM iodoacetamide in 50 mM NH4HCO3 in the dark for 30 min. Subsequently, 12.5 ng/μl trypsin in 25 mM NH4HCO3 was added to saturate and cover gel slices. The enzymatic reaction was carried out overnight at 37 °C. After digestion, the peptides were extracted from the gel pieces by adding 5% formic acid at 37 °C for 30 min. The gel pieces were spun down and the liquid collected. The extraction was repeated twice. Finally, the extracted peptide mixture was lyophilized to dryness and dissolved in 10 μl 0.1% formic acid.

HPLC and Mass Spectrometry for Proteomics—The peptide mixtures were analyzed by nanoHPLC (Eksigent Technologies, Inc., Dublin, CA) coupled to an LTQ-Orbitrap (Thermo Fisher Scientific, Bremen, Germany) hybrid mass spectrometer. Peptides were separated on a C18 column at a 200 nl/min flow rate by using a gradient of 2–98% solvent B (98% acetonitrile in water, 0.1% formic acid) over 130 min. The eluates were on-line electrospayed into the mass spectrometer via a nanoelectrospray ion source (Thermo Fisher Scientific, San Jose, CA). The LTQ-Orbitrap was running in positive ion, top five data-dependent acquisition mode. For full scans in the Orbitrap, the target ion value was 1,000,000, and the maximal injection time was 500 ms at a resolution of r = 60,000 at m/z 400. The MS full scan range was 380–1600 m/z.

Peptide and Protein Identification—The Orbitrap raw files were searched against a concatenated forward and reversed IPI-mouse database version 3.46 containing 55,272 protein sequences. Peak picking and searches were performed by Extract_msn and the SEQUEST search engine (8), respectively, both of which included in the Bioworks software (version 3.3.1; Thermo Fisher Scientific). Searches used trypsin as enzyme and allowed for up to one missed cleavage. The 14N database search was performed by using the following parameters: 20ppm mass tolerance for the MS scan, 1Da for the MS/MS scan, fixed carbamidomethylation for cysteine, and variable oxidation for methionine. The 15N database search was executed using above parameters plus 15N amino acid masses and an additional hypothetical 1Da variable modification for arginine and lysine residues (9). The SEQUEST results were filtered by using peptide XCorr >1.9 for 1+ charged ions, >2.7 for 2+ charged ions, >3.5 for 3+ or above charged ions, and DeltaCN >0.08. False discovery rates were calculated using the MAYU software (10) based on the number of peptides matching reversed database entries and the number of peptides matching forward database entries. Redundancy and ambiguity in protein identifications were addressed by grouping proteins, which could not be distinguished based on the identified peptides. Each group consists of proteins with identical sets of identified peptides. Proteins, whose peptides were subsets of other proteins’ peptides, were removed. This resulted in a minimal list of
proteins for all observed peptides, following “Occam’s law of parsimony.” Proteins within a group were treated equally. Thus, in the following when we refer to a “protein” we mean the group it belongs to. Furthermore, proteins were required to have at least two identified peptides with distinct sequences.

**Protein Quantification and Significance Analysis**—Relative quantification of the peptide pair signals was performed with the ProRata software using default parameters (11). Briefly, for each peptide ion chromatograms were extracted based on peak area for both labeled and unlabeled isotope envelopes according to the amino acid sequence. The peak profile of both chromatograms was used to determine abundance ratio and signal-to-noise ratio of the peptide. ProRata removes peptides with insufficient signal-to-noise ratio and proteins with less than two quantified peptides. ProRata protein abundance ratio estimation is based on a probabilistic model of the peptide ratio distributions. This model was used to calculate HAB/LAB protein ratios and to estimate their statistical significance as outlined in the following. We combined biological replicates and calculated protein ratios across groups based on a separate ProRata tool for downstream analysis (combine.exe, available at http://code.google.com/p/prorata/). Briefly, for each protein, peptide ratios of biological replicates were combined within groups (HAB/NAB and LAB/NAB) to calculate a profile likelihood of the protein abundance ratio for each group. Profile likelihoods of both groups were subsequently combined via cross-correlation to get a probabilistic estimate of the indirect HAB/LAB abundance ratio. For each protein, a p value for the null hypothesis of no differential expression (i.e., log2 ratio = 0) was derived from the profile likelihood by means of a likelihood ratio test. Protein ratios were considered to be statistically significant based on a p value threshold of 0.05, which was corrected for multiple testing by the procedure of Benjamini-Hochberg (12). Additionally, a protein fold change (determined as the maximum likelihood estimate) of at least two was required for true “differential expression.” This eliminates a number of protein hits, which would be significant based on their p value, but have a fold change too small to be biologically meaningful.

**Western Blot Verification**—Relative expression levels for several proteins were further analyzed by Western blot. These proteins were selected based on commercial antibody availability and their relevance for psychiatric disorders. Protein mixtures with equal protein content (30 μg) were first resolved by SDS-PAGE. Subsequently, the separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA). After incubation with antigen-specific antibodies (anti-carbonic anhydrase sc-17244, anti-

**RESULTS**

**Proteomic Analyses**—For the sensitive quantitative proteomic analysis of the HAB/LAB mouse model we have used tissue specimens from NAB animals metabolically labeled with the stable isotope $^{15}$N as reference. Brain and plasma proteins obtained at PND 56 showed a $^{15}$N incorporation that was greater than 90% (5). The EPM behavioral test demonstrated phenotypes that were consistent for all three animal lines, HAB, LAB, and NAB, used in this study (5).

In previous proteomic analyses we had identified Glx1 as a biomarker for trait anxiety by two dimensional polyacrylamide gel electrophoresis (2-DE) in both brain tissue and blood cells (1). In order to confirm the differential Glx1 expression between HAB and LAB mice and validate the metabolic labeling-based proteomic method employed in the present study, relative Glx1 expression levels were assessed between HAB and LAB mice (Fig. 1). In hippocampus, Glx1 shows a fivefold higher expression level in LAB compared with HAB mice. These results confirm our earlier findings obtained by 2-DE (1).
In the following, we present data for the in-depth quantitative proteomic analyses of hippocampal and plasma proteins from the trait anxiety mouse model. The hippocampus was chosen because it represents a brain region that has been implicated in the pathobiology of a number of psychiatric disorders including anxiety and is therefore an important source for gaining insights into dysfunctional molecular pathways. Because of its availability the specimen of choice for biomarker analyses in the clinical laboratory is plasma.

From the hippocampal cytosolic fraction 18,594 distinct peptides and 2956 protein groups, from the hippocampal microsome fraction 19,749 peptides and 3289 groups, and from plasma 5049 peptides and 1297 groups were identified by tandem mass spectrometry after filtering the data using the criteria mentioned above. We used the MAYU (10) software (version 1.06) to estimate false discovery rates (FDR) on the peptide-spectrum match level and the protein level. Because of the relatively stringent filtering criteria, peptide-spectrum match FDRs were estimated as 0.6%, 0.7%, and 1.2% for cytosol, microsome, and plasma, respectively. We required proteins to have at least two peptide identifications with distinct sequences, which removes the majority of decoy proteins. Thus, corresponding protein level FDRs were similarly low in case of cytosol and microsome and amounted to 0.8% and 0.6%, respectively. For plasma, however, we observed an increased protein FDR of 8.2%. Closer inspection of the data revealed that the majority of decoy proteins had been identified in the 15N database search only. Because of the higher complexity of the 15N isotopeologue patterns, a higher number of false positives is to be expected in the 14N search compared with the 15N search. To estimate the impact of this bias, we recalculated the protein FDR of plasma excluding proteins, which had been identified in the 15N search only. The FDR dropped to an acceptable level of 3%, demonstrating that the majority of dubious protein hits is caused by 15N-only identifications. Because the plasma data is not used in any downstream analysis, we did not adjust the filtering criteria. Instead, to allow for a better judgment of the reliability of protein identifications, we provide for all supplementary data in addition to the total number of unique identifications the number of 14N peptide identifications with unique sequences per protein. Since almost all of the reported proteins have at least one 14N identification we expect the impact of increased false positives resulting from 15N searches to be negligible. In total, 1576 proteins were quantified, of which 312 were found to be differentially expressed (≥twofold change, ≥2 unique peptides, corrected p value < 0.05) in the hippocampal cytosolic fraction between HAB and LAB mice (supplemental Table S1). For the hippocampal microsome fraction, a total of 1349 proteins were quantified, of which 206 were found to be differentially expressed (supplemental Table S2). In plasma 383 proteins were quantified, of which 58 were found to be differentially expressed (supplemental Table S3). These numbers only reflect proteins identified and quantified in both 14N-HAB/15N-NAB and 14N-LAB/15N-NAB comparisons.

Expression level differences for three biomarker candidates, carbonic anhydrase 2 (CA2), transthyretin (TTR), and serum amyloid P-component (SAP), were further verified by Western blot analyses (Fig. 2). Based on previous reports these proteins are of particular interest because they have been implicated to play a role in psychiatric disorder pathobiology (19–22).

**Metabolomic Analyses**—The great complexity of the brain and cellular heterogeneity even within a defined brain section like the hippocampus makes the identification of metabolite biomarkers in this tissue difficult. We therefore restricted our metabolomic analyses to HAB and LAB mouse plasma specimens. From plasma 265 metabolites were detected by GC-MS analysis of which 86 have known chemical structures. The concentrations of 15 plasma metabolites of known identity differed significantly (p value < 0.05) between the HAB and LAB mice (supplemental Table S1). For the hippocampal cytosolic fraction, a total of 1349 proteins were quantified, of which 206 were found to be differentially expressed (supplemental Table S2). In plasma 383 proteins were quantified, of which 58 were found to be differentially expressed (supplemental Table S3). These numbers only reflect proteins identified and quantified in both 14N-HAB/15N-NAB and 14N-LAB/15N-NAB comparisons.

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Fig. 2. Relative protein quantification in HAB/LAB mice. A, D, G, Eluted chromatographic profiles for carbonic anhydrase 2 (CA2, IPI00121534) tryptic peptide AVQQPDGLAVLGIFLK, transthyretin (TTR, IPI00127560) tryptic peptide TAESGELHGLTTDEK, serum amyloid P-component (SAP, IPI00309214) tryptic peptide GRDNELLIYKEK, respectively. The peak areas are used for the $^{14}$N/$^{15}$N signal quantification. In all cases $^{15}$N-labeled NAB proteins were used as a reference and either mixed with unlabeled HAB or LAB material; B, E, H, Western blot analyses of CA2, TTR and SAP protein levels; C, F, I, Western blot protein band density quantification for CA2 ($p = 0.0025$), TTR ($p < 0.0001$) and SAP ($p = 0.0008$), respectively. In all cases the Western blot analyses confirm the relative protein expression level data obtained by mass spectrometry.
LAB lines (supplemental Table S4). Among these are two inositol isomers. Whereas HAB mice showed a higher level for allo-inositol (p value = 0.011), LAB mice had higher levels of its isomer myo-inositol (p value = 0.002) (Fig. 3). Myo-inositol has been shown to have antidepressant and anxiolytic activities in both humans and animals (23–28). Also of interest is the finding that several energy metabolism related metabolites, including amino acids, cholesterol, fumarate, and malate, were found at different levels between the two lines. Moreover, the key excitatory neurotransmitter, glutamate, was expressed at higher levels in HAB compared with LAB mice (p value = 0.000002) (Fig. 3).

**KEGG and GO Analyses**—The correlations between differential hippocampal protein expression and KEGG and “GO cellular component” are shown in Fig. 4. The pathways and categories enriched with proteins from the noncentral bins are of greatest interest because they indicate a significant protein expression level difference between HAB and LAB animals. In the following we highlight those identified pathways that have particular relevance for psychiatric phenotypes.

Based on the KEGG and GO analyses, proteins responsible for inositol phosphate metabolism (p value = 0.0016), phosphatidylinositol signaling (p value = 3.93E-5), and phosphatidylinositol binding (p value = 0.0436) were expressed at higher levels in LAB compared with HAB mice. Inositol has been associated with psychiatric disorders, especially bipolar disorder pathobiology in a number of studies. Based on our results the “phosphatidylinositol signaling system” is apparently dysregulated in HAB mice exemplified by an alteration of a number of protein and metabolite levels.

Ubiquitin Mediated Proteolysis (p value = 2.62E-4), ubiquitin-protein ligase activity (p value = 0.001), proteasome (p value = 6.22E-5), and “ubiquitin-specific protease activity” (p value = 0.04) proteins were found at lower levels in HAB compared with LAB mice. This finding is in accordance with previous reports indicating that ubiquitin ligase may act as an anxiety suppressor (29). Further supporting the significance of proteasome-ubiquitin mediated protein degradation for the anxiety phenotype is the fact that “long-term depression” pathway associated proteins were enriched in the first bin (p value = 0.02). An association of the two pathways has been previously reported (30). On the other hand, proteins relevant for “long-term potentiation” (p value = 0.04) were expressed at higher levels in HAB mice, which supports previous electrophysiology data performed in our laboratory (31).

The KEGG analyses also revealed proteins involved in pathways related to energy metabolism, including glycolysis (p value = 6.31E-13), pyruvate metabolism (p value = 4.4E-9), and the TCA cycle (p value = 2.02E-8). In addition, GO analyses demonstrated that electron carrier activity (p value = 0.04), aerobic respiration (p value = 0.024), electron transport chain (p value = 0.04), “acetyl-CoA metabolic process” (p value = 0.006), and the mitochondria (p value = 3.65E-10), are affected in HAB mice. Fumarate and malate, two major intermediates of the TCA cycle, were found at higher levels in HAB mice. The conversion from fumarate to malate is catalyzed by fumarate hydratase, which, in agreement with the metabolite data, was found at an elevated expression level in HAB mice. The same trend was observed for all the other major enzymes that are part of the TCA cycle, including citrate synthase, aconitase, isocitrate dehydrogenase and malate dehydrogenase (Fig. 5A), indicating a major alteration of this pathway. Furthermore, GO analyses revealed that proteins involved in peroxiredoxin activity (p value = 0.005), oxidoreductase activity (p value = 6.62E-4) and oxidation reduction (p value = 1.89E-7) were more abundant in HAB mice, suggesting an important role for oxidative stress in anxiety etiology.

GO analyses further demonstrated that proteins relevant to the synapse (p value = 0.01), stress fiber (p value = 3.19E-4), and “neuron projection” (p value = 0.01) were expressed at higher levels in LAB mice. Proteins relevant to “neurotransmitter catabolic process” (p value = 0.02), were found expressed at higher levels in HAB mice, indicating a crucial role of neurotransmission in anxiety.

**Subnetwork Enrichment**—The above in silico analyses focused on predefined KEGG pathways. To identify general network hotspots, we also conducted a subnetwork enrichment analysis. For this purpose we grouped differentially expressed proteins into small interaction maps, allowing the identification of small nonstatic pathways sharing a high correlation with the phenotype. These analyses indicated the involvement of a number of networks highly enriched with
proteins and metabolites differentially expressed between HAB and LAB mice. A significant number of subnetworks were related to the Ras/Raf/MEK/ERK pathway. The central entities in these subnetworks either inhibit the Ras/Raf/MEK/ERK pathway, including PD 98059 (p-value/11005 1.05484E-22) (32), genistein (p-value/11005 3.32622E-17) (33), and wortmannin (p-value/11005 5.64E-13) (34), or are themselves part of the pathway, including MAP2K1 (p-value/11005 3.49E-6), MAPK1 (p-value/11005 2.3E-5), MAPK3 (p-value/11005 1.8E-4), and MAPK8 (p-value/11005 0.001). The identification of both subnetwork types suggests an involvement of this pathway in anxiety-related behavior. In addition, the GO analyses (see above) also demonstrated that the proteins involved in protein serine/threonine kinase activity (p-value/11005 3.98E-4) and small GTPase regulator activity (p-value/11005 0.02), which are quite relevant for the Ras/Raf/MEK/ERK pathway, were expressed at higher levels in LAB mice.

**DISCUSSION**

The pathogenesis of psychiatric disorders remains elusive, and there is growing evidence that several neural circuits and brain pathways are affected. For the characterization of these pathways we have used a systems biology analysis based on both proteomic and metabolic data from a robust trait anxiety network entities are highly enriched with proteins and metabolites differentially expressed between HAB and LAB mice (Fig. 5B). Dexamethasone is a synthetic cortisol and a modulator of the hypothalamus-pituitary-adrenal (HPA) axis whose dysfunction has been implicated to play a major role in depression (42–46).

Glycinergic synaptic transmission (p-value/11005 1.84E-9) is also of particular importance for psychiatric disorders (Fig. 5C). Glycine is an inhibitory neurotransmitter in the spinal cord and brainstem and has been shown to have a key function in the regulation of locomotor behavior (47–50) and beneficial effects in the treatment of depression (51, 52). In addition, proteins relevant for myelin maintenance (p-value/11005 4.73E-8) (Fig. 5C) were also enriched.
mouse model (1). A quantitative proteomic approach that involves metabolic labeling of mice with stable isotopes (4) has enabled us to identify and quantify a large number of proteins in a high throughput manner. Differentially expressed proteins were further interrogated with regard to pathways they are involved in. The resulting protein and metabolite interaction maps suggest several biological processes and pathways to be affected in the genetic predisposition to extremes in trait anxiety. Whether these pathways are causative or the result of distinct psychiatric endophenotypes is unknown at the present time. Be that as it may, the HAB/LAB mouse model used in the present study faithfully represents a number of endophenotypic aspects also found in patients afflicted with anxiety disorders.

A major finding of our analyses implicates the inositol pathway to be critically involved in the anxiety phenotype with several proteins and metabolites that are part of phosphatidylinositol signaling having altered expression levels. Inositol has been shown in several reports to have anxiolytic effects and lithium, a well known mood stabilizer used for treating bipolar disorder, is believed to exert its therapeutic effects through the inositol pathway by decreasing intracellular myo-inositol concentrations (53). Our findings of different myo- and allo-inositol levels in the trait anxiety mouse model lend further support to the relevance of the inositol pathway for psychiatric phenotypes including anxiety.

Oxidative stress has been found to be involved in the pathogenesis of neurological diseases, including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, and stroke (54) as well as psychiatric disorders (55–61). Human studies on panic disorder and obsessive-compulsive disorder also suggest an involvement of oxidative stress in anxiety (62–64). Oxidative stress is caused by altered mitochondrial energy pathways leading to abundant reactive oxidative stress compounds. It is therefore not surprising that TCA cycle enzyme and metabolite levels were found to be significantly different between HAB and LAB mice. In studies by others it was also shown that stress-induced anxiety in mice leads to elevated levels for a number of TCA cycle intermediates (65) that can result in excessive oxidative damage. Results from other animal and patient studies also support these findings (60, 66).

A dysfunctional HPA axis that has lost its ability of negative feedback inhibition is considered a hallmark in depression and anxiety disorders (43, 45, 46). The HPA axis and glucocorticoids regulate neuronal survival, neurogenesis, memory, FIG. 5. TCA cycle pathways and biological subnetworks enriched with proteins and metabolites. A, TCA cycle; enzymes and metabolites labeled in red are expressed at higher levels in HAB compared with LAB mice. B, Subnetwork related to dexamethasone; C, subnetworks related to glycnergic synaptic transmission and myelin maintenance. (because of the limited image resolution the protein and metabolite names are not legible; a higher resolution figure is available in the electronic file.)
and emotions (67). Excess glucocorticoids may impair or even damage the hippocampus, which may initiate and maintain a hypercortisolemic state found in certain cases of depression (68). Our data show that a significant number of entities relevant to dexamethasone are altered between HAB and LAB mice, indicating an involvement of the HPA axis in anxiety.

An elevated excitatory or decreased inhibitory neurotransmission is frequently observed in depressive and anxious patients (69–71). Anxiety disorder treatment is targeting neurotransmitter pathways using either benzodiazepines or selective serotonin reuptake inhibitors (SSRIs) (72). In our metabolomic analyses the major excitatory neurotransmitter, glutamate, which binds to the N-methyl-D-aspartate (NMDA) receptor was found at higher levels in HAB compared with LAB mouse plasma. This result is consistent with previous findings in patients with depression where higher levels of glutamate in blood, CSF, and certain brain regions were found (69–71, 73, 74). NMDA receptor antagonists have antidepressant effects and are promising alternatives to monoamine-based agents for the treatment of depression and anxiety (75–78). Our study has also identified a number of candidate proteins associated with glycineric synaptic transmission (Fig. 5C). Both glycine and GABA are essential inhibitory neurotransmitters in the central nervous system. Although glycine’s involvement in psychiatric disorders is less understood than that of GABA’s, the co-localization and release of GABA and glycine are widespread in inhibitory neurons of the brain and spinal cord. GABA acts as a co-agonist to modify the response of glycine receptors (79). In this regard studies have found that glycine exerts inhibitory effects in certain brain areas, resulting in significant anxiety relief (80, 81).

The Ras/Raf/MEK/ERK pathway is a signal transduction pathway involved in metazoan development. It controls many biological processes, including metabolic processes, cell cycle, cell migration, and cell shape as well as cell proliferation and differentiation (82). In contrast to its relevance in cancer, the importance of the Ras/Raf/MEK/ERK pathway in psychiatric disorders is still poorly understood. The corticotropin-releasing hormone receptor, which is part of the HPA axis discussed above, exerts its function through the activation of the Ras/Raf/MEK/ERK pathway in psychiatric disorders is still poorly understood. The corticotropin-releasing hormone receptor, which is part of the HPA axis discussed above, exerts its function through the activation of the Ras/Raf/MEK/ERK pathway (83–85). In the present study, a number of enriched sub-networks were found to be relevant for the Ras/Raf/MEK/ERK pathway. Furthermore, inhibition of the Ras/Raf/MEK/ERK pathway by the MEK inhibitor U0126 was found to decrease the depression-like behavior in both wild-type and mutant mice, indicating an involvement of this pathway in psychiatric disorders (41).

Our data further support the notion of anxiety as a polygenic trait caused by multiple gene products, each providing a minor contribution to the phenotype, which is additionally shaped by environmental influences. Such gene-by-environment interactions can induce persistent functional changes in neuronal pathways that underlie variation in anxiety-related behavior and vulnerability to anxiety. This flow of information from DNA to the anxiety phenotype includes a variety of proteins, metabolites and other molecular biomarkers (86). Which of them are causally related to the phenotype is unknown at the present time.

In summary, our -omics data implicate a number of proteins, metabolites and pathways that corroborate previous findings on psychiatric disorder pathobiology. They lend further support to the validity of the trait anxiety mouse model for further experiments with the goal to verify candidate biomarkers in patients afflicted with anxiety disorders and test new medications for their treatment.

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This article contains supplemental Tables S1 to S4.

REFERENCES

1. Krömer, S. A., Kessler, M. S., Miltay, D., Birg, I. N., Bunck, M., Czibere, L., Panhuysen, M., Plütz, B., Deussing, J. M., Hölsbofer, F., Landgraf, R., and Turk, C. W. (2005) Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. J. Neurosci. 25, 4375–4384
2. Ditzen, C., Jastorff, A. M., Kessler, M. S., Bunck, M., Teplytska, L., Erhardt, A., Krömer, S. A., Varadarajulu, J., Targosz, B. S., Sayan-Ayata, E. F., Hölsbofer, F., Landgraf, R., and Turk, C. W. (2006) Protein biomarkers in a mouse model of extremes in trait anxiety. Mol. Cell. Proteomics 5, 1914–1920
3. Plomin, R., Haworth, C. M., and Davis, O. S. (2009) Common disorders are quantitative traits. Nat. Rev. Genet. 10, 872–878
4. Wu, C. C., MacCoss, M. J., Howell, K. E., Matthews, D. E., and Yates, J. R., 3rd (2004) Metabolic labeling of mammalian organisms with stable isotopes for quantitative proteomic analysis. Anal. Chem. 76, 4951–4959
5. Reiter, L., Claassen, M., Schirmpf, S. P., Jovanovic, M., Schmidt, A., Buhmann, J. M., Hengartner, M. C., and Aebersold, R. (2009) Protein identification false discovery rates for very large proteomics data sets generated by tandem mass spectrometry. Mol. Cell. Proteomics 8, 2405–2417
6. Pan, C., Kora, G., McDonald, W. H., Tabb, D. L., VerBerkmoes, N. C., Hurst, G. B., Pelletier, D. A., Samatová, N. F., and Hettich, R. L. (2006) ProRata: A quantitative proteomics program for accurate protein abundance ratio estimation with confidence interval evaluation. Anal. Chem. 78, 4265–4270
7. Cox, B., and Emili, A. (2006) Tissue subcellular fractionation and protein extraction for use in mass-spectrometry-based proteomics. Nat. Protoc. 1, 1872–1878
8. Eng, J. K., McCormack, A. L., and Yates, J. R. (1994) An Approach to Correlate Tandem Mass-Spectral Data of Peptides with Amino-Acid-Sequences in a Protein Database. J. Am. Soc. Mass Spectrom. 5, 976–980
9. Zhang, Y., Webhofer, C., Reckow, S., Filiou, M. D., Zhang, Y., Maccarrone, G., Reckow, S., Bunck, M., Heumann, H., Turk, C. W., Landgraf, R., and Hambisch, B. (2009) Stable isotope metabolic labeling with a novel N-enriched bacteria diet for improved proteomic analyses of mouse models for psychopathologies. PLoS ONE 4, e7821
10. Reiter, L., Claassen, M., Schirmpf, S. P., Jovanovic, M., Schmidt, A., Buhmann, J. M., Hengartner, M. C., and Aebersold, R. (2009) Protein identification false discovery rates for very large proteomics data sets generated by tandem mass spectrometry. Mol. Cell. Proteomics 8, 2405–2417
11. Pan, C., Kora, G., McDonald, W. H., Tabb, D. L., VerBerkmoes, N. C., Hurst, G. B., Pelletier, D. A., Samatová, N. F., and Hettich, R. L. (2006) ProRata: A quantitative proteomics program for accurate protein abundance ratio estimation with confidence interval evaluation. Anal. Chem. 78, 4265–4270
33. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., and Fukami, Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. J. Biol. Chem. 262, 5592–5595

34. Wymann, M. P., Burguillare-Leva, G., Zvelibeli, M. J., Pirolo, L., Vanhaecke-broek, B., Waterfield, M. D., and Panayotou, G. (1996) Wortmannin inactivates phosphoinositide 3-kinase by covalent modification of Lys-802, a residue involved in the phosphate transfer reaction. Mol. Cell. Biol. 16, 1722–1733

35. Dwivedi, Y., Razvi, H. S., Roberts, R. C., Conley, R. C., Tamminga, C. A., and Pandey, G. N. (2001) Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. J. Neurochem. 77, 916–928

36. Feng, P., Guan, Z., Yang, X., and Fang, J. (2003) Impairments of ERK signal transduction in the brain in a rat model of depression induced by neonatal exposure of clomipramine. Brain Res. 991, 195–205

37. Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Kedves, A. T., Duman, R. S., and Taylor, J. R. (2008) Region-specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. Biol. Psychiatry 63, 353–359

38. Qi, X., Lin, W., Li, J., Li, H., Wang, W., Wang, D., and Sun, M. (2008) Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress. Neuropsychobiology. 31, 278–285

39. Tirosh, E., Tardito, D., Haskin, I., Moraschi, S., Pruner, P., Gennarelli, M., Racagni, G., and Pololi, M. (2004) Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. Neuropsychopharmacology 29, 1831–1840

40. Qi, X., Lin, W., Wang, D., Pan, Y., Wang, W., and Sun, M. (2009) A role for the extracellular signal-regulated kinase signal pathway in depression-like behavior. Behav. Brain Res. 190, 203–209

41. Todrovic, C., Sherrin, T., Pitts, M., Hippiel, C., Rayner, M., and Spiess, J. (2009) Suppression of the MEK/ERK signaling pathway reverses depression-like behaviors of CRF2-deficient mice. Neuropsychopharmacology 34, 1416–1426

42. de Kloet, E. R., Joëls, M., and Holsboer, F. (2005) Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 483–487

43. Handwerger, K. (2008) Differential patterns of HPA activity and reactivity in adult posttraumatic stress disorder and major depressive disorder. Harv. Rev. Psychiatry 17, 184–205

44. Yu, S., Holsboer, F., and Almeida, O. F. (2008) Neuronal actions of glucocorticoids: focus on depression. J. Steroid Biochem. Mol. Biol. 108, 300–309

45. Müller, M. B., and Holsboer, F. (2006) Mice with mutations in the HPA-axis: a new model for panic disorder. Am. J. Psychiatry 153, 1219–1221

46. Brittain, C., Rujescu, D., Giegling, I., Turck, C. W., Holsboer, F., Bullmore, E. T., Middleton, L., Merlo-Pich, E., Alexander, R. C., and Muglia, P. (2006) A synthetic inhibitor of the mitogen-activated protein-kinase cascade. Proc. Natl Acad. Sci. U. S. A. 103, 1526–1531

47. Kaur, C., and Ling, E. A. (2008) Antioxidants and neuroprotection in the treatment of depression. Drug Discov. Today 13, 1040–1048

48. Mann, J. J., Marangell, L. B., Goetz, R. R., and Gorman, J. M. (1999) Low levels of transthyretin in the CSF of depressed patients. Biol. Psychiatry 45, 257–258

49. Hashimoto-Gotoh, T., Iwabe, N., Tsujimura, A., Takao, K., and Miyakawa, T. (2003) Methylglyoxal-mediated anxiolysis involves increased protein modification and elevated expression of glyoxalase 1 in the brain. J. Neurochem. 83, 1240–1251

50. Xu, T. L., and Gong, N. (2010) Glycine and glycine receptor signaling in hippocampal neurons: Diversity, function and regulation. Prog. Neurobiol. 91, 348–361

51. Weinberg, M. H. (1945) Aminoacetic acid (glutamic acid) in the treatment of depression. J. Nervous Mental Dis. 102, 601–610

52. Javitt, D. C. (2004) Glutamate as a therapeutic target in psychiatric disorders. Mol. Psychiatry 9, 1104–1115

53. Boucard, A., Holsboer, F., and Almeida, O. F. (2008) Neuronal actions of glucocorticoids: focus on depression. J. Steroid Biochem. Mol. Biol. 108, 300–309

54. Kirsch, J. (2006) Glycerin transmission. Cell Tissue Res. 326, 535–540

55. Xu, T. L., and Gong, N. (2010) Glycine and glycine receptor signaling in hippocampal neurons: Diversity, function and regulation. Prog. Neurobiol. 91, 348–361

56. Weinberg, M. H. (1945) Aminoacetic acid (glutamic acid) in the treatment of depression. J. Nervous Mental Dis. 102, 601–610

57. Javitt, D. C. (2004) Glutamate as a therapeutic target in psychiatric disorders. Mol. Psychiatry 9, 1104–1115

58. Harwood, A. J. (2005) Lithium and bipolar mood disorder: the insulitodepletion hypothesis revisited. Mol. Psychiatry 10, 117–126

59. Kaur, C., and Ling, E. A. (2008) Antioxidants and neuroprotection in the adult and developing central nervous system. Curr. Med. Chem. 15, 3068–3080

60. Bouquet, J., Rammal, H., and Soulainman, R. (2009) Oxidative stress and anxiety: Relationship and cellular pathways. Oxid. Med. Cell. Longev. 2, 63–67

61. Adibhatla, R. M., and Hatcher, J. F. (2010) Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid. Redox. Signal. 12, 125–169

62. Wood, S. J., Yücel, M., Pantelis, C., and Berk, M. (2009) Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress. Ann. Neuropsychopharmacology 34, 1416–1426

63. de Kloet, E. R., Joëls, M., and Holsboer, F. (2005) Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 483–487

64. Handwerger, K. (2008) Differential patterns of HPA activity and reactivity in adult posttraumatic stress disorder and major depressive disorder. Harv. Rev. Psychiatry 17, 184–205

65. Yu, S., Holsboer, F., and Almeida, O. F. (2008) Neuronal actions of glucocorticoids: focus on depression. J. Steroid Biochem. Mol. Biol. 108, 300–309

66. Müller, M. B., and Holsboer, F. (2006) Mice with mutations in the HPA-axis: a new model for panic disorder. Am. J. Psychiatry 153, 1219–1221

67. Brittain, C., Rujescu, D., Giegling, I., Turck, C. W., Holsboer, F., Bullmore, E. T., Middleton, L., Merlo-Pich, E., Alexander, R. C., and Muglia, P. (2006) A synthetic inhibitor of the mitogen-activated protein-kinase cascade. Proc. Natl Acad. Sci. U. S. A. 103, 1526–1531

68. Kaur, C., and Ling, E. A. (2008) Antioxidants and neuroprotection in the adult and developing central nervous system. Curr. Med. Chem. 15, 3068–3080

69. Bouquet, J., Rammal, H., and Soulainman, R. (2009) Oxidative stress and anxiety: Relationship and cellular pathways. Oxid. Med. Cell. Longev. 2, 63–67

70. Adibhatla, R. M., and Hatcher, J. F. (2010) Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid. Redox. Signal. 12, 125–169

71. Wood, S. J., Yücel, M., Pantelis, C., and Berk, M. (2009) Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress. Ann. Neuropsychopharmacology 34, 1416–1426

Trait Anxiety Pathways

Molecular & Cellular Proteomics 10.12
58. Do, K. G., Cabungcal, J. H., Frank, A., Steullet, P., and Cuenod, M. (2009) Redox dysregulation, neurodevelopment, and schizophrenia. Curr. Opin. Neurobiol. 19, 220–230

59. Tyolec, A., Jarzab, A., Strzyjeka-Zimmer, M., and Wójcicka, A. (2007) [Stress oxidative in schizophrenia]. Pol. Merkur Lekarski 23, 74–77

60. Andreazza, A. C., Kauer-Sant’anna, M., Frey, B. N., Bond, D. J., Kapczinski, F., Young, L. T., and Yatham, L. N. (2008) Oxidative stress markers in bipolar disorder: a meta-analysis. J. Affect. Disord. 111, 135–144

61. Ng, F., Berk, M., Dean, O., and Bush, A. I. (2008) Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int. J. Neuropsychopharmacol. 11, 851–876

62. Ersan, S., Bakir, S., Erdal Ersan, E., and Dogan, O. (2006) Examination of free radical metabolism and antioxidant defence system elements in patients with obsessive-compulsive disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 30, 1039–1042

63. Kuloglu, M., Atmaca, M., Tezcan, E., Gecici, O., Tunckol, H., and Ustundag, B. (2002) Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. Neuropsychobiology 46, 27–32

64. Kuloglu, M., Atmaca, M., Tezcan, E., Ustundag, B., and Bulut, S. (2002) Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. Neuropsychobiology 46, 186–189

65. Thurston, J. H., and Hauhart, R. E. (1989) Effect of momentary stress on brain energy metabolism in weaning mice: apparent use of lactate as cerebral metabolic fuel concomitant with a decrease in brain glucose utilization. Metab. Brain Dis. 4, 177–186

66. Jou, S. H., Chiu, N. Y., and Liu, C. S. (2009) Mitochondrial dysfunction and psychiatric disorders. Chang. Gung Med. J. 32, 370–379

67. Herbert, J., Goodyer, I. M., Grossman, A. B., Hastings, M. H., de Kloet, E. R., Lightman, S. L., Lupien, S. J., Roozendaal, B., and Seckl, J. R. (2006) Do corticosteroids damage the brain? J. Neuroendocrinol. 18, 393–411

68. Nestler, E. J., Barrot, M., DiLeone, R. J., Eisich, A. J., Gold, S. J., and Monteggia, L. M. (2002) Neurobiology of depression. Neuron 34, 13–25

69. Küçükibrahimoglu, E., Saygin, M. Z., Calişkan, M., Kaplan, O. K., Unsal, C., and Gören, M. Z. (2009) The change in plasma GABA, glutamine and glutamate levels in fluoxetine- or S-citalopram-treated female patients with major depression. Eur. J. Clin. Pharmacol. 65, 571–577

70. Sanacora, G., Gueorguieva, R., Epperson, C. N., Wu, Y. T., Appel, M., Rothman, D. L., Krystal, J. H., and Mason, G. F. (2004) Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. Arch. Gen. Psychiatry 61, 705–713

71. Bhagwagar, Z., Wylezinska, M., Jezzard, P., Evans, J., Ashworth, F., Sule, A., Matthews, P. M., and Cowen, P. J. (2007) Reduction in occipital cortex gamma-aminobutyric acid concentrations in medication-free recovered unipolar depressed and bipolar subjects. Biol. Psychiatry 61, 806–812

72. Gingrich, J. A. (2005) Oxidative stress is the new stress. Nat. Med. 11, 1281–1282

73. Kim, J. S., Schmid-Burgk, W., Claus, D., and Komhuber, H. H. (1982) Increased serum glutamate in depressed patients. Arch. Psychiatr. Nervenkr. 232, 299–304

74. Levine, J., Panchalingam, K., Rapoport, A., Gershon, S., McClure, R. J., and Pettegrew, J. W. (2000) Increased cerebrospinal fluid glucose levels in depressed patients. Biol. Psychiatry 47, 586–593

75. Hashimoto, K. (2009) Emerging role of glutamate in the pathophysiology of major depressive disorder. Brain Res. Rev. 61, 105–123

76. Skolnick, P., Popik, P., and Trullas, R. (2009) Glutamate-based antidepressants: 20 years on. Trends Pharmacol. Sci. 30, 563–569

77. Boyce-Rustay, J. M., and Holmes, A. (2006) Genetic inactivation of the NMDA receptor NR2A subunit has anxiolytic- and antidepressant-like effects in mice. Neuropsychopharmacology 31, 2405–2414

78. Berton, O., and Nestler, E. J. (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat. Rev. Neurosci. 7, 137–151

79. Lu, T., Rubio, M. E., and Trussell, L. O. (2008) Glycinergic transmission shaped by the corelease of GABA in a mammalian auditory synapse. Neuron 57, 524–535

80. Young, A. B., ZUKIN, S. R., and Snyder, S. H. (1974) Interaction of benzodiazepines with central nervous glycine receptors: possible mechanism of action. Proc. Natl. Acad. Sci. U.S.A. 71, 2246–2250

81. Chojnacka-Wójcik, E., Klodzinska, A., and Plic, A. (2001) Glutamate receptor ligands as anxiolytics. Curr. Opin. Investig. Drugs 2, 1112–1119

82. Schlessinger, J. (2000) Cell signaling by receptor tyrosine kinases. Cell 103, 211–225

83. Hauger, R. L., Risbrough, V., Brauns, O., and Dautzenberg, F. M. (2006) Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets. CNS Neurol. Disord. Drug Tar-gets 5, 453–479

84. Hillhouse, E. W., and Grammatopoulos, D. K. (2006) The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. Endocr. Rev. 27, 260–286

85. Sananbenesi, F., Fischer, A., Schrick, C., Spiess, J., and Radulovic, J. (2003) Mitogen-activated protein kinase signalling in the hippocampus and its modulation by corticotropin-releasing factor receptor 2: a possible link between stress and fear memory. J. Neurosci. 23, 11436–11443

86. Mackay, T. F., Stone, E. A., and Ayroles, J. F. (2009) The genetics of quantitative traits: challenges and prospects. Nat. Rev. Genet. 10, 565–577

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