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Convergent functional genomics of anxiety disorders: translational identification of genes, biomarkers, pathways and mechanisms

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Anxiety disorders are prevalent and disabling yet understudied from a genetic standpoint, compared with other major psychiatric disorders such as bipolar disorder and schizophrenia. The fact that they are more common, diverse and perceived as embedded in normal life may explain this relative oversight. In addition, as for other psychiatric disorders, there are technical challenges related to the identification and validation of candidate genes and peripheral biomarkers. Human studies, particularly genetic ones, are susceptible to the issue of being underpowered, because of genetic heterogeneity, the effect of variable environmental exposure on gene expression, and difficulty of accrual of large, well phenotyped cohorts. Animal model gene expression studies, in a genetically homogeneous and experimentally tractable setting, can avoid artifacts and provide sensitivity of detection. Subsequent translational integration of the animal model datasets with human genetic and gene expression datasets can ensure cross-validatory power and specificity for illness. We have used a pharmacogenomic mouse model (involving treatments with an anxiogenic drug—yohimbine, and an anti-anxiety drug—diazepam) as a discovery engine for identification of anxiety candidate genes as well as potential blood biomarkers. Gene expression changes in key brain regions for anxiety (prefrontal cortex, amygdala and hippocampus) and blood were analyzed using a convergent functional genomics (CFG) approach, which integrates our new data with published human and animal model data, as a translational strategy of cross-matching and prioritizing findings. Our work identifies top candidate genes (such as *FOS*, *GABBR1*, *NR4A2*, *DRD1*, *ADORA2A*, *QKI*, *RGS2*, *PTGDS*, *HSPA1B*, *DYNLL2*, *CCKBR* and *DBP*), brain–blood biomarkers (such as *FOS*, *QKI* and *HSPA1B*), pathways (such as cAMP signaling) and mechanisms for anxiety disorders—notably signal transduction and reactivity to environment, with a prominent role for the hippocampus. Overall, this work complements our previous similar work (on bipolar mood disorders and schizophrenia) conducted over the last decade. It concludes our programmatic first pass mapping of the genomic landscape of the triad of major psychiatric disorder domains using CFG, and permitted us to uncover the significant genetic overlap between anxiety and these other major psychiatric disorders, notably the under-appreciated overlap with schizophrenia. *PDE10A*, *TAC1* and other genes uncovered by our work provide a molecular basis for the frequently observed clinical co-morbidity and interdependence between anxiety and other major psychiatric disorders, and suggest schizo-anxiety as a possible new nosological domain.
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Introduction

'Worry is a thin stream of fear trickling through the mind. If encouraged, it cuts a channel into which all other thoughts are drained.'

–Arthur Somers Roche

Anxiety disorders are prevalent and disabling. Approximately 30 million people are affected with anxiety disorders in United States[1,2] and the 12-month prevalence rate is estimated to be 18.1%.[3] Anxiety disorders, under DSM classification, include generalized anxiety disorder (GAD), panic disorder, specific phobias, post-traumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD). They can be grouped into those without an obvious external trigger (GAD, panic disorder), those with an obvious external trigger (PTSD, phobias) and those that are more of a mixed picture, like OCD. Anxiety disorders are often co-morbid with other psychiatric disorders such as depression, bipolar disorder, schizophrenia and substance abuse.[4,5] Phenomenologicaly, anxiety disorders seem to have in common an increased reactivity to the environment, driven by uncertainty and fear of perceived threats.[6] Stress is a common trigger and/or exacerbator.

Despite their prevalence and clinical impact, anxiety disorders are understudied from a genetic standpoint, compared with other major psychiatric disorders. Twin, adoption and familial studies have suggested a role for

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heritability in anxiety disorders.[7,8] Human genetic linkage studies have identified some susceptibility loci.[9–11] Genetic association studies have identified polymorphisms in genes such as corticotropin-releasing hormone (CRH),[12] glutamate transporter (SLC1A1),[13] adenosine A2a receptor (ADORA2A),[14] regulator of G-protein signaling 2 (RGS2),[15,16] delta-aminolevulinate dehydratase (ALAD),[17] dynein light chain 2 (DYNLL2)[17] and others as possibly involved in anxiety disorders, but with limited reproducibility. There are few published human postmortem brain gene expression studies to date on anxiety and related disorders.[18,19]

To overcome this suboptimal state of affairs, we employed a comprehensive convergent functional genomics (CFG)[20–23] approach as a way of identifying and prioritizing candidate genes and blood biomarkers for anxiety disorders, as we did in our previous work on bipolar disorder,[24–28] schizophrenia[29,30] and alcoholism.[31] As a first step, we used drug effects on gene expression in mice in key brain regions for anxiety (prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HIP)),[32] as well as blood (BLD), as a way to tag genes that may have pathophysiological relevance. We then cross-matched and integrated that gene-level data with multiple other lines of evidence (genetic and gene expression) from human studies and other animal model studies (Figure 1).

For our mouse brain and blood gene expression studies, we used an agonist drug, which induces symptoms of anxiety (yohimbine),[33–35] and a gold standard antagonist drug, which is used to treat anxiety disorders (diazepam)[36,37] (Figure 1). From the range of doses of the drugs that had been reported in the literature to have our desired behavioral effects, we chose doses at the low end of the range, to minimize potential supraphysiological dosing artifacts and side-effects. We also employed a behavioral readout to make sure the drugs were absorbed and doing what they were supposed to do (Figure 2).

Changes in gene expression in response to each of the two drugs, yohimbine and diazepam, would be of interest in and of themselves, in terms of candidate gene generation and CFG. However, not all genes that show changes in expression in response to either of the drugs are necessarily germane to the pathophysiology of anxiety and related disorders. It is likely that some of the gene expression changes have to do

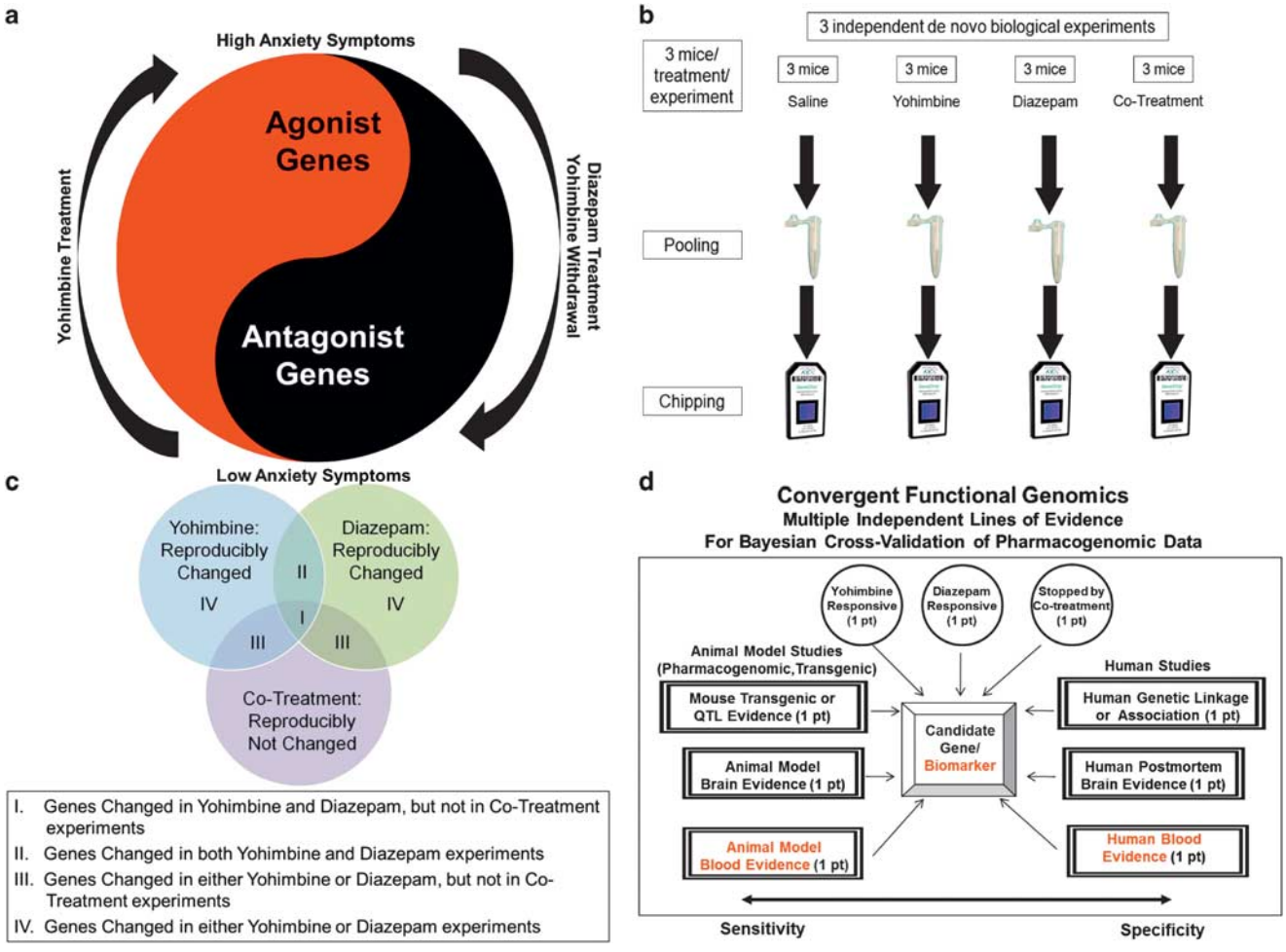
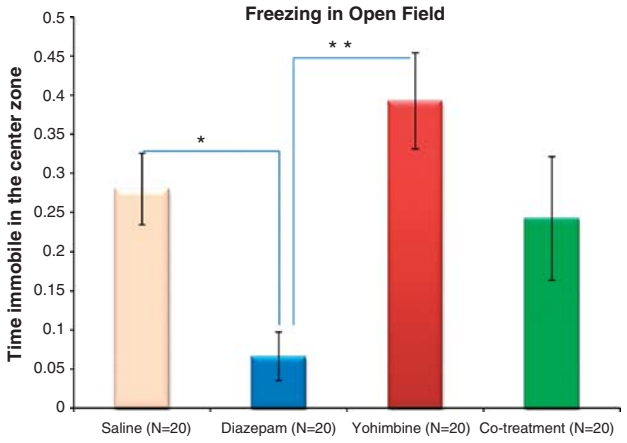


Figure 1 Design of experiments and data analysis. (**a**) Pharmacological treatment paradigm. (**b**) Experimental design. (**c**) Venn diagram categorizing genes changed by the various drug treatments, and their classification into categories I, II, III and IV. (**d**) Multiple converging independent internal and external lines of evidence for cross-validation and prioritization of findings.



Comparison	P-value
Saline vs. Diazepam	0.000221569
Saline vs. Yohimbine	0.075053656
Saline vs. Co-treatment	0.340754175
Diazepam vs. Yohimbine	0.000028318
Diazepam vs. Co-treatment	0.023987402
Yohimbine vs. Co-treatment	0.071028122

Figure 2 Behavioral correlates of diazepam and yohimbine treatment—time immobile in center zone. Analysis of mouse open field video-tracking behavioral phenotype data from 15 to 30 min after drug injections. Ratio of resting time in the center zone vs total time spent in the center zone. This measure reflects freezing behavior, an anxiety-driven phenomenon. Yohimbine increases freezing, diazepam reduces it, and co-treatment does not have an effect. One-tail *t*-tests are depicted. (*) Statistically significant. The difference between diazepam and yohimbine is highly statistically significant (**).

with other effects of the drugs, particularly their individual side-effects. We reasoned, first, that genes that change in expression in response to both drugs are more likely to be involved in the core pathophysiology we are modeling, and are higher probability candidate genes. Second, co-treatment with the two drugs, one an anxiogenic, and the other one an anxiolytic, could arguably show interference effects, and some of the genes that would be changed by single drug treatment would be 'nipped in the bud' and show no changes in expression in response to co-treatment. Those genes would also be deemed higher probability candidate genes than the genes that still change during co-treatment.

As external cross-validators, for each gene changed in expression in our pharmacogenomics studies, we used six independent lines of evidence in our CFG analyses (Figure 1d). First, we assessed if there was any published genetic evidence—human genetic evidence of association with anxiety, or at least if it mapped to a linkage locus that had been implicated in anxiety disorders. We also looked at mouse transgenic or quantitative trait loci (QTL) studies relevant to anxiety. Second, we assessed if there was any published gene expression evidence in brain or blood in anxiety disorders, from human studies and, more broadly, from other animal models of anxiety.[38] These external lines of evidence suffer from the obvious drawback of being constrained by what has been published so far, limiting novelty, and to the inherent biases and limitations of those particular lines of work.

According to Bayesian theory, an optimal estimate results from combining previous information with new evidence. Although we cannot exclude that some of the candidate genes we have identified are false positives because of potential biological or technical limitations of the methodology and approach we employed, the higher the number of independent lines of evidence (i.e. the higher the CFG score), the lower the likelihood of that being the case. The CFG scoring is arguably a reasonable compromise between specificity and sensitivity, between focus and broadness.

Our approach identifies and prioritizes an extensive series of candidate genes, some of which have already been reported using various related treatments or paradigms, as well as many others which are novel. Moreover, the coalescence of the candidate genes into pathways and mechanisms is of particular importance and opens new directions. Finally, we compared our results with our previous similar work in bipolar disorder,[25,26] schizophrenia[29] and alcoholism,[31] and were able to analyze the significant genetic overlap between anxiety and these other disorders, providing a molecular basis for the frequently observed clinical co-morbidity.

Materials and methods

Yohimbine and diazepam treatments. All experiments were performed with male C57/BL6 mice, 8–12 weeks of age, obtained from Jackson Laboratories (Bar Harbor, ME, USA), and acclimated for at least 2 weeks in our animal facility (Indiana University School of Medicine LARC) on reverse light cycle (1000 to 2200 hours) before any experimental manipulation. All experiments were conducted at the same time of day—between 1400 and 1600 hours. Mice were treated by intraperitoneal injection with single-dose of yohimbine (1 mg kg^{-1}), diazepam (0.3 mg kg^{-1}), a combination of yohimbine and diazepam (1 and 0.3 mg kg^{-1}), or control (vehicle) solution only. The control solution, which was also used to dissolve the drugs, consisted of 0.325% Tween 80 in 0.9% phosphate-buffered saline and alcohol (EtOH) at a final concentration of 10 μl ml^{-1} EtOH.

Behavioral studies. A SMART II Video Tracker system (San Diego Instruments, San Diego, CA, USA) was used to track movement of mice under normal light immediately after drug administration. After injection, mice were placed in the lower right-hand corner of one of four adjacent, 41_41_34-cm^3 enclosures. Mice had no physical contact with other mice during testing. Each enclosure has nine pre-defined areas, that is, center area, corner areas and wall areas. After an initial 15 min of adaptation, measures of locomotor activity were obtained from the second half (15 min) of the total 30-min time recorded immediately after injection of the drugs, with a focus on behavior in the open field center area.

Gene expression studies. Three independent *de novo* biological experiments, performed at different times, were used for gene expression studies. Each experiment

consisted of three mice per treatment condition, for a total of nine mice per condition across the three experiments (Figure 1b). Brain and blood from the same *de novo* experiment were used for microarray studies.

Microdissection. Twenty-four hours after drug administration, mice were sacrificed by cervical dislocation. The brains of the mice were harvested, stereotactically sliced and hand micro-dissected using Paxinos mouse anatomical atlas coordinates, to isolate anatomical regions of interest—PFC, AMY and HIP.[25,27,29] Tissues were flash frozen in liquid nitrogen and stored at −80 °C until future processing for RNA extraction and gene expression analyses. Approximately 1 ml of blood/mouse was collected in PAXgene blood RNAcollection tubes (PreAnalytix, Qiagen, San Jose, CA, USA). The PAXgene tubes were stored at 4 °C overnight, and then at −80 °C until future processing for RNA extraction.

Microarrays. We used Mouse Genome 430 2.0 arrays (Affymetrix, Santa Clara, CA, USA). The GeneChip Mouse Genome 430 2.0 Array contain 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. Microarrays used in each independent experiment were derived from the same manufacturing lot.

RNA extraction and hybridization. For each brain region (PFC, AMY and HIP) and blood, equal amounts of total RNA extracted from tissue samples were pooled within each biological experiment (three mice per treatment group), and then used for labeling and microarray assays.

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 micro-dissected mouse brain regions. For the whole mouse blood RNA extraction, PAXgene blood RNA extraction kit (PreAnalytiX, a Qiagen/BD Biosciences, San Jose, CA, USA) was used, followed by GLOBINclearTM–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, Palo Alto, CA, USA). The quantity and quality of total RNA was also independently assessed by 260 nm ultraviolet absorption and by 260/280 ratios, respectively. Starting material of total RNA labeling reactions was kept consistent within each independent microarray experiment.

Standard Affymetrix protocols were used to reverse transcribe the messenger RNA and generate biotinlylated complementary RNA (http://www.affymetrix.com/support). The amount of complementary RNA 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 within acceptable limits.

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 each drug treatment, using its corresponding saline vehicle treatment 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 $P < 0.00025$ was used. Only transcripts that were called present in at least one of the two samples (saline vehicle or drug) intra-experiment, and that were reproducibly changed in the same direction in at least two out of three independent experiments, were analyzed further.

Gene identification. The identities of transcripts were established using NetAFFX (Affymetrix). Probe-sets that did not have a known gene were labeled 'EST' and their accession numbers kept as identifiers.

CFG analyses

Databases. We have established in our laboratory (Laboratory of Neurophenomics, Indiana University School of Medicine, www.neurophenomics.info) 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.[21] Only the findings deemed significant in the primary publication, by the study investigators, using their particular experimental design and thresholds, are included in our databases. These constantly updated large databases have been used in our CFG cross-validation (Figure 1).

Human genetic evidence (association, linkage). To designate convergence for a particular gene, the gene had to have published evidence of association or linkage for anxiety disorders, including PTSD, OCD, panic disorder and phobias. For linkage, the location of each gene was obtained through GeneCards (http://www.genecards.org), and the sex averaged cM location of the start of the gene was then obtained through http://compgen.rutgers.edu/old/map-interpolator/. For convergence, per our previously published criteria,[25] the start of the gene had to map within 10 cM of the location of a marker linked to the disorder.

Human gene expression evidence (postmortem brain, blood). Information about genes was obtained and imported in our databases searching the primary literature with PubMed (http://ncbi.nlm.nih.gov/PubMed), using various combinations of keywords (gene name, anxiety, stress, phobia, panic, PTSD, OCD, human, brain, postmortem, blood, lymphocytes, fibroblasts). Convergence was deemed to occur for a gene if there were published human postmortem brain data (or, rarely, blood and other tissue

data) showing changes in expression of that gene in tissue from patients with anxiety and related disorders.

Mouse genetic evidence (transgenic, QTL). To search for mouse genetic evidence—QTL or transgenic—for our candidate genes, we utilized the MGI_3.54—Mouse Genome Informatics (http://www.informatics.jax.org). (Jackson Laboratory) and used the search 'Genes and Markers' form to find QTL or transgenic for Mammalian Phenotype Ontology category 'abnormal emotion/affect behavior', which includes the following sub-categories: abnormal fear/anxiety-related behavior, abnormal response to novelty and aggression-related behavior. 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 brain and blood gene expression evidence. For animal model brain and blood gene expression evidence, we have used in addition to our own data, published reports from the literature, curated in our databases.

CFG analysis scoring. Only genes reproducibly changed in expression in the same mouse tissue (PFC, AMY, HIP and blood), in the same direction, in two out of three independent experiments, were analyzed further. The three internal lines of evidence (pharmacological treatments—changed in yohimbine, changed in diazepam, no change in co-treatment) were scored with 1 point each. The six external cross-validating lines of evidence (three animal models, 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 (Figure 1d). The 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 $3+6=9$.

The more lines of evidence, that is, the more times a gene shows up as a positive finding across independent studies, platforms, methodologies and species, the higher its CFG score (Figure 1d). This is very similar conceptually to a Google PageRank algorithm, in which the more links to a page, the higher it comes up on the search prioritization list.[23] Human and animal model, genetic and gene expression, data sets were integrated and tabulated. 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, this simple scoring system, where the different independent lines of evidence are weighted equally, and more of the lines of evidence are related to gene expression rather than genetics, arguably provides a good separation and prioritization of genes and blood biomarkers that are changed in expression and disease relevant, our stated focus.

Pathway analyses. Ingenuity 8.5 (Ingenuity Systems, Redwood City, CA, USA) was used to analyze the biological roles, including top canonical pathways, of the candidate genes resulting from our work (Table 5, Supplementary Table S2), as well as employed to identify genes in our data sets that are the target of existing drugs (Supplementary Table S4). GeneGo (Thompson Reuters) was used to analyze the disease categories of the genes identified (Table 7, Supplementary Table S3).

Results

Our pharmacogenomics animal model displays a behavioral readout consistent with the drugs having an impact and their intended effects—anxiogenic for yohimbine, anxiolytic for diazepam and mitigation of effects for co-treatment (Figure 2).

We have a relatively large number of genes changed in expression in the mouse tissues examined (three brain regions and blood) (Table 1).

To start with, we have grouped the mouse model gene expression changes into categories I–IV, as described in Figure 1c and Table 1. We reasoned that genes that are category I genes, which are changed in expression by both the agonist and antagonist, as well as not changed ('nipped in the bud') by co-treatment, are more likely to be involved in the core biology of anxiety disorders rather than be pleiotropic effects/side-effects of the drugs we used. Of note, the HIP and the blood have a relatively greater proportion of category I genes than the other brain regions (Table 1), suggesting an important role in anxiety disorders for the HIP, and a possible peripheral effect/biomarker readout for the blood.

For CFG scoring, each internal pharmacological line of evidence (changed in expression by yohimbine, changed by diazepam and not changed by co-treatment) was scored separately, along with each of the six external lines of evidence (three from animal model studies, and three from human studies), resulting in a maximum possible CFG score of 9 (Figure 1). Genes that have a CFG score of 4 or above, i.e. they have at least one full external line of evidence in addition to the maximal possible score of 3 from the internal evidence, were prioritized and shown in Table 2 and Figure 3. The average CFG score for the top candidate genes (Table 2) was again highest for HIP (4.4), followed by AMY (4.37), PFC (4.34) and blood (4.26). The relative role of HIP in anxiety

Table 1 Number of genes reproducibly changed in different regions, classified by categories I–IV

	Category I (% of total)	*Category II*	*Category III—diazepam*	*Category III—yohimbine*	*Category IV—diazepam*	*Category IV—yohimbine*	*Total*
Prefrontal cortex	4 (3.9%)	3	29	32	15	19	102
Amygdala	4 (3.2%)	12	46	32	11	20	125
Hippocampus	32 (10.2%)	10	56	194	11	11	314
Blood	54 (11.0%)	41	246	100	36	16	492

Table 2 Top candidate genes for anxiety

PFC Gene symbol/name	*PFC YH*	*PFC DZ*	*PFC Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG Score*
DRD1/dopamine receptor D1	I		NC	(I) Anxiety[69] (I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Increased anxiety-related response			5q35.2 (Association) PD[45]	5.0
ATP2B1/ATPase, Ca++ transporting, plasma membrane 1	D		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]				(I) Chronic stress[48]	12q21.33 (Linkage) PD[71]	4.5
CRIM1/cysteine rich transmembrane BMP regulator 1 (chordin like)	D		NC	(D) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			2p22.3 (Association) PD[72]	4.5
ENC1/ectodermal-neural cortex 1	D		NC	(D) DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior		(I) PD lymphocyte[73]		4.5
HSPA1B/heat shock protein 1B		I	NC	(I) DBP ST PFC[70]				(I) Chronic stress[48]	6p21.33 (Linkage) Neuroticism[74] PD[75]	4.5
IGF2/insulin-like growth factor 2	D	D		(D) Chronic restraint stress[76,77]				(I) PTSD lymphocyte[78]	11p15.5 (Linkage) OCD in males[79]	4.5
PENK/proenkephalin	I		NC	(D) Anxiety[80] (D) Stress[81] (I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Increased anxiety-related response			8q12.1 Anxiety (Linkage)[82]	4.5
RASD2/RASD family, member 2	I		NC	(I) DBP ST AMY[70]		(Transgenic) Increased anxiety-related response			22q12.3 (Linkage) PD[75]	4.5
RBBP4/retinoblastoma binding protein 4	I		NC	(D) DBP ST AMY (D) DBP ST PFC[70]				(D) Psychological stress[83]	1p35.1 (Linkage) Neuroticism[74]	4.5
SGK1/serum/glucocorticoid regulated kinase1	I	I	NC	(I) Stress[81]		(QTL) Abnormal emotion/affect behavior				4.5
DBP/D site albumin promoter-binding protein	I		NC	(I) Anxiety[69] (D) Stress[81]		(Transgenic) Stress[70]				4.0
DRD2/dopamine receptor 2	I			(D) DBP ST PFC[70]		(Transgenic) Decreased anxiety-related response			11q23.2 (Association) Stress/ depression[84] PD[45] PTSD[85] Anxiety/social phobia[86]	4.0
GAS5/growth arrest-specific 5		D	NC	(D) Chronic restraint stress[76] (D) DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior			1q25.1 (Linkage) PD[87]	4.0
GSK3B/glycogen synthase kinase 3 beta	D		NC	(D) Stress[88] (I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Behavioral despair				4.0

Table 2 Continued

PFC *Gene symbol/name*	*PFC YH*	*PFC DZ*	*PFC Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG Score*
PDE10A/phosphodiesterase 10A	I		NC	(D) DBP ST PFC[70]		(Transgenic) Decreased exploration in new environment				4.0
ZFP36L2/zinc-finger protein 36, C3H type-like 2	D		NC			(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	2p21 (Linkage) PD[89]	4.0
AMY *Gene symbol/name*	*AMY YH*	*AMY DZ*	*AMY Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human Brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG score*
GABBR1/gamma-aminobutyric acid (GABA) B receptor, 1	I		NC	(I) Anxiety[90]		(Transgenic) Impaired passive avoidance behavior, increased anxiety-related response		(D) PTSD[40]	6p22.1 (Association) OCD[41]	6.0
FOS/FBJ osteosarcoma oncogene		I	NC	(I) Anxiety[91] (I) Stress[81] (I)DBP ST AMY[70]		(Transgenic) Decreased anxiety-related response		(I) PTSD[40] (I) Stress[83,92]	14q24.3(Linkage) OCD[93,94]	5.5
ADORA2A/adenosine A2a receptor		I		(D)DBP ST PFC[70]		(Transgenic) Increased anxiety-related response		(I) Chronic stress[48]	22q11.23 (Association) Caffeine-induced anxiety[95] PD[46,47,96]	5.0
QKI/quaking homolog, KH domain RNA binding	I		NC	(I)DBP ST AMY[70]		(Transgenic) Abnormal response to novel object		(D) Chronic stress[48]		5.0
HSPA8/heat shock protein 8	D	D		(I) Stress[97]				(D) Chronic stress[48]	11q24.1 (Linkage) Neuroticism[98]	4.5
PTPRD/protein tyrosine phosphatase, receptor type, D	D	D	NC	(D) Stress[81] (I)DBP ST AMY[70]					9p23 (Linkage) OCD[94,99,100]	4.5
RBBP4/retinoblastoma-binding protein 4	I		NC	(D)DBP ST AMY; (D)DBP ST PFC[70]				(D) Stress[83]	1p35.1 (Linkage) Neuroticism[74]	4.5
DGKG/diacylglycerol kinase, gamma		D	NC	(D)DBP ST AMY[70]		(QTL) Abnormal fear/ anxiety-related behavior increased freezing fear response			3q27.3 (Linkage) OCD[101]	4.0
DYNLL2/dynein light chain LC8-type 2		I	NC	(D) Anxiety[102]					17q22 (Association) Anxiety[17]	4.0

Table 2 Continued

AMY ***Gene symbol/name***	***AMY YH***	***AMY DZ***	***AMY Co-TX***	***Animal models brain evidence***	***Animal models blood evidence***	***Animal genetic (QTL/transgenic) evidence***	***Human Brain evidence***	***Human blood evidence***	***Human genetic (linkage/ association) evidence***	***CFG score***
GAS5/growth arrest-specific 5	D	D		(D) Chronic restraint stress[76] (D)DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior			1q25.1 (Linkage) PD[87]	4.0
GNAS/(guanine nucleotide-binding protein, alpha stimulating) complex locus		I	NC		(D) DBP ST Blood[70]	(Transgenic) Abnormal response to new environment				4.0
HSPA13/heat shock protein 70 family, member 13	I		NC				(D)PTSD[19]	(I) Chronic stress[48]		4.0
HSPA4/heat shock protein 4	I	I	NC					(D) Stress[83]		4.0
KCNMA1/potassium large conductance calcium-activated channel, subfamily M, alpha member 1	I		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			10q22.3 (Linkage) OCD in autism[103]	4.0
NUDT21/nudix (nucleoside diphosphate-linked moiety X)-type motif 21	D		NC	(I) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			16q12.2 (Linkage) Social phobia[11]	4.0
PAFAH1B1/platelet-activating factor acetylhydrolase, isoform 1b, subunit 1		I	NC	(D) DBP ST AMY[70]				(D) Chronic stress[48]		4.0
RORB/RAR-related orphan receptor beta	I		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Decreased aggression				4.0
SFRS18/splicing factor, arginine/ serine-rich 18	D	D	NC	(I) PFC DBP ST[70]						4.0
SYNGR1/synaptogyrin 1	I	I		(D)Stress[104] (D) Shock avoidance learning (fear)[105]		(QTL) Abnormal emotion/affect behavior			22q13.1 (Linkage) PD[75]	4.0

HIP ***Gene symbol/name***	***HIP YH***	***HIP DZ***	***HIP Co-TX***	***Animal models brain evidence***	***Animal models blood evidence***	***Animal genetic (QTL/transgenic) evidence***	***Human brain evidence***	***Human blood evidence***	***Human genetic (linkage/ association) evidence***	***CFG Score***
FOS/FBJ osteosarcoma oncogene	I	I	NC	(I) Anxiety[91] (I) Stress[81] (I) DBP ST AMY[70]		(Transgenic) Decreased anxiety-related response		(I) PTSD[40] (I) Stress[83,92]	14q24.3 (Linkage) OCD[93,94]	6.5
NR4A2/nuclear receptor subfamily 4, group A, member 2	I	I	NC	(D) Anxiety[69] (D) Stress[81]		(Transgenic) Behavioral despair impaired passive avoidance behavior			2q24.1 (Linkage) PD[89]	5.5
CAMK2D/calcium/calmodulin-dependent protein kinase II, delta	I	I	NC	(I) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			4q26 (Linkage) Autism/OCD[103]	5.0

Table 2 Continued

HIP Gene symbol/name	HIP YH	HIP DZ	HIP Co-TX	Animal models brain evidence	Animal models blood evidence	Animal genetic (QTL/transgenic) evidence	Human brain evidence	Human blood evidence	Human genetic (linkage/ association) evidence	CFG Score
DRD1/dopamine receptor D1	I		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Increased anxiety-related response			5q35.2 (Association) PD[45]	5.0
EGR1/early growth response 1	I		NC	(I) Anxiety[90] (I) Anxiety and stress[106] (D) Stress[81] (I) DBP ST AMY[70]		(Transgenic) Decreased aggression		(I) Leukocytes high lonely individuals (social epidemiological risk factor)[107]		5.0
GUCY1A3/guanylate cyclase 1, soluble, alpha 3	I	I	NC	(I) Anxiety[108] (D) DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior			4q32.1 (Linkage) Anxiety[10]	5.0
HOMER1/homer homolog 1 (Drosophila)	I	I		(D) Anxiety[69] (D) Stress[109] (D) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior		(D) PTSD[40]	5q14.1 (Linkage) Anxiety[10]	5.0
MEF2C/myocyte enhancer factor 2C	I		NC	(I) DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	5q14 (Linkage) Anxiety[10]	5.0
PTGDS/prostaglandin D2 synthase (brain)	I		NC	(I) Anxiety[102] (D) Anxiety[97] (D) Stress[81]		(Transgenic) Decreased aggression			9q34.3 (Association) Anxiety[17]	5.0
RGS2/regulator of G-protein signaling 2	I		NC	(D) Anxiety[69] (D) DBP ST PFC[70]		(Transgenic) Decreased aggression			1q31.2 (Association) Anxiety[15] PTSD[16]	5.0
ARPP21/cyclic AMP-regulated phosphoprotein, 21	I	I	NC	(D) DBP ST PFC[70]					3p22.3 (Linkage) Anxiety/PD[82]	4.5
ATP2B1/ATPase, Ca++ transporting, plasma membrane 1	D		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]				(I) Chronic stress[48]	12q21.33 (Linkage) PD[71]	4.5
CDKN1A/cyclin-dependent kinase inhibitor 1A (P21)	I		NC	(I) PPI of startle[110]				(I) Chronic stress[48]	6p21.31 (Linkage) Neuroticism[74]	4.5
DNAJB1/DnaJ (Hsp40) homolog, subfamily B, member 1	I	I	NC	(D) Primates stress-induced[111]		(QTL) Abnormal emotion/affect behavior				4.5
EGR2/early growth response 2	I	I	NC	(I) Anxiety/ depression[112] (I) Stress[109]					10q21.3 (Linkage) PD[113]	4.5
HSPA1B/heat shock protein 1B	I		NC	(I) DBP ST PFC[70]				(I) Chronic stress[48]	6p21.33 (Linkage) PD[75] Neuroticism[74]	4.5
PENK/preproenkephalin	I	I		(D) Anxiety[80] (D) Stress[81] (I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Increased anxiety-related response			8q12.1 (Linkage) Anxiety[82]	4.5

Table 2 Continued

HIP Gene symbol/name	*HIP YH*	*HIP DZ*	*HIP Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG Score*
SERPINI1/serine (or cysteine) peptidase inhibitor, clade I, member 1	I		NC	(I) Anxiety[90] (D) Primates stress-induced[111] (D) DBP ST AMY[70]		(Transgenic) Abnormal anxiety-related response			3q26.1 (Linkage) Agoraphobia[87] Simple phobia[114]	4.5
TAC1/tachykinin 1	I		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Decreased anxiety-related response, increased coping response			7q21.3 (Linkage) PD[115]	4.5
VGLL3/vestigial-like 3 (Drosophila)	D	I	NC	(D) DBP ST AMY[70]					3p12.1 (Linkage) PD[82] Neuroticism[98]	4.5
ADCY8/adenylate cyclase 8	I		NC	(I) Harm avoidance behavior[116]		(Transgenic) Decreased anxiety-related response				4.0
APAF1/apoptotic peptidase-activating factor 1	I	I	NC					(D) Chronic stress[48] (I) Stress[83]		4.0
BTG2/B-cell translocation gene 2, anti-proliferative	D	I				(QTL) Abnormal fear/ anxiety-related behavior		(I) High lonely individuals (social epidemiological risk factor), leukocyte[107]	1q32.1 (Linkage) PD[71]	4.0
CCKBR/cholecystokinin B receptor	I		NC	(I) Anxiety[117,118]					11p15.4 (Association) PD[96,119–121]	4.0
DGKG/diacylglycerol kinase, gamma	D		NC	(D) DBP ST AMY[70]		(QTL) Abnormal fear/ anxiety-related behavior			3q27–q28 (Linkage) OCD[93]	4.0
DYRK1A/dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a	D	D	NC			(Transgenic) Decreased anxiety-related response				4.0
FOXP2/forkhead box P2	I		NC	(I) DBP ST AMY; (D) DBP ST PFC[70]		(Transgenic) Decreased exploration in new environment				4.0
HPCAL1/hippocalcin-like 1	I	I	NC	(D) DBP ST AMY[70]						4.0
KCNF1/potassium voltage-gated channel, subfamily F, member 1	I	I	NC	(I) DBP ST PFC[70]						4.0
KCNIP2/Kv channel-interacting protein 2	D	D	NC	(D) DBP ST PFC[70]						4.0
KCTD12/potassium channel tetramerization domain containing 12	D		NC	(D) DBP ST AMY[70]				(D) Chronic stress[48]		4.0

Table 2 Continued

HIP Gene symbol/name	HIP YH	HIP DZ	HIP Co-TX	Animal models brain evidence	Animal models blood evidence	Animal genetic (QTL/transgenic) evidence	Human brain evidence	Human blood evidence	Human genetic (linkage/ association) evidence	CFG Score
LEFTY1/left right determination factor 1	D	D	NC			(QTL) Abnormal emotion/affect behavior			1q42.12 (Linkage) PD[75]	4.0
LHX9/LIM homeobox protein 9	D		NC	(D) DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior			1q31.3 (Linkage) PD[71]	4.0
LPL/lipoprotein lipase	D		NC	(D) DBP ST AMY (D) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			8p21.3 (Linkage) Anxiety[122]	4.0
MNDA/myeloid cell nuclear differentiation antigen	D		NC			(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	1q23.1 (Linkage) Anxiety[122] OCD[101]	4.0
NELL2/NEL-like 2 (chicken)	D		NC	(D) Stress[81] (I) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			12q12 (Linkage) PD[123]	4.0
PIP4K2C/phosphatidylinositol-5-phosphate 4-kinase, type II, gamma	D		NC	(I) Anxiety[109]		(QTL) Abnormal emotion/affect behavior			12q13.3 (Linkage) PD[123]	4.0
PRKG2/protein kinase, cGMP-dependent, type II	I	I	NC			(QTL) Abnormal emotion/affect behavior			4q21.21 (Linkage) Anxiety[10]	4.0
RGS4/regulator of G-protein signaling 4	I	I		(I) Anxiety[109] (D) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			1q23.3 (Linkage) Anxiety[122] OCD[93]	4.0
RORB/RAR-related orphan receptor beta	I		NC	(I) DBP ST AMY (D) DBP ST PFC[70]		(Transgenic) Decreased aggression				4.0
SPINK8/serine peptidase inhibitor, Kazal type 8	D	D	NC	(D) DBP ST AMY[70]						4.0
SPP1/secreted phosphoprotein 1	I	I	NC			(QTL) Abnormal emotion/affect behavior			4q22.1 (Linkage) Anxiety[10]	4.0
STMN1/stathmin 1	I		NC		(D) DBP ST Blood[70]				1p36.11 (Association) Fear and anxiety[124]	4.0
TIPARP/TCDD-inducible poly(ADP-ribose) polymerase		I	NC			(QTL) Abnormal emotion/affect behavior		(I) Peripheral blood monocytes chronic stress[48]	3q25.31 (Linkage) Agoraphobia simple phobia[114]	4.0
TRHR/thyrotropin-releasing hormone receptor	I	I	NC	(I) Stress[81] (D) DBP ST AMY[70]						4.0

Table 2 Continued

BLOOD (BLD) Gene symbol/name	*BLD YH*	*BLD DZ*	*BLD Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG score*
FOS/FBJ osteosarcoma oncogene	D	I	NC	(I) Anxiety[91] (I) Stress[81] (I) DBP ST AMY[70]		(Transgenic) Decreased anxiety-related response		(I) PTSD[40] (I) Stress[83,92]	14q24.3 (Linkage) OCD[93,94]	6.5
HSPA8/heat shock protein 8	I	I	NC	(I) stress[97]				(D) Chronic stress[48]	11q24.1 (Linkage) Neuroticism[98]	5.5
IL1B/interleukin 1 beta	D		NC	(I) PD[109,125]				(I) High lonely individuals (social epidemiological risk factor)[107]	2q13 (Association) Anxiety[126]	5.0
QKI/quaking	I		NC	(I) DBP ST AMY[70]		(Transgenic) Abnormal response to novel object		(D) Chronic stress[48]		5.0
RGS2/regulator of G-protein signaling 2		I	NC	(D) Anxiety[69] (D) DBP ST PFC[70]		(Transgenic) Decreased aggression			1q31.2 (Association) Anxiety[15] PTSD[16]	5.0
CNP/2′,3′-cyclic nucleotide 3′ phosphodiesterase	I	I	NC	(D) DBP ST PFC[70] (I) Anxiety[90]		(QTL) Abnormal emotion/affect behavior				4.5
CORO1A/coronin, actin-binding protein 1A	I	I	NC					(D) Chronic stress[48]	16p11.2 (Linkage) PD[127] Social phobia[11]	4.5
HSPA1B/heat shock protein 1B		I	NC	(I) DBP ST PFC[70]				(I) Chronic stress[48]	6p21.33 (Linkage) Neuroticism[74] PD[75]	4.5
IL2RG/interleukin 2 receptor, gamma chain	I	I	NC			(QTL) Abnormal emotion/affect behavior		(I) PTSD[128]		4.5
LY6E/lymphocyte antigen 6 complex, locus E	I	I	NC	(D) Stress[81]		(QTL) Abnormal emotion/affect behavior				4.5
MDH1/malate dehydrogenase 1, NAD (soluble)	I	I	NC	(D) Stress[97]		(QTL) Abnormal emotion/affect behavior				4.5
S100A10/S100 calcium binding protein A10 (calpactin)	I		NC	(I) Anxiety[102] (D) Stress[97] (D) DBP ST AMY[70]		(Transgenic) Abnormal depression-related behavior			1q21.3 (Linkage) Anxiety[122]	4.5
SNCA/synuclein, alpha		D	NC	(I) Anxiety[102]; (D) DBP ST AMY[70]	(D) DBP ST Blood[70]				4q22.1 (Linkage) Anxiety[10]	4.5
ANP32E/acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	NC	D	NC	(D) DBP ST PFC[70]		(QTL) Abnormal emotion/affect behavior			1q21.2 (Linkage) Anxiety[122]	4.0
CALM1/calmodulin 1		I	NC	(D) Anxiety[129]				(D) Chronic stress[48]		4.0

Table 2 Continued

BLOOD (BLD) Gene symbol/name	*BLD YH*	*BLD DZ*	*BLD Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG score*
CHCHD2/coiled-coil-helix domain containing 2	I	I	NC			(QTL) Abnormal emotion/affect behavior			7p11.2 (Linkage) OCD[101]	4.0
CSF2RB/colony-stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	D	I				(QTL) Abnormal emotion/affect behavior		(D) PTSD[78]	22q12.3 (Linkage) PD[113] Harm avoidance (anxiety proneness)[130]	4.0
CSF3R/colony-stimulating factor 3 receptor (granulocyte)		I	NC			(QTL) Abnormal emotion/affect behavior		(I) Stress peripheral blood cells[83] (I) Stress[131]	1p34.3 (Linkage) Neuroticism[74]	4.0
CTNNB1/catenin (cadherin associated protein), beta 1		I	NC	(I) Anxiety[129]				(I) Stress[131]		4.0
CYBA/cytochrome b-245, alpha polypeptide		I	NC	(D) Stress[81]			(D) PTSD[19]			4.0
FAIM3/Fas apoptotic inhibitory molecule 3	I	I	NC			(QTL) Abnormal emotion/affect behavior			1q32.1 (Linkage) PD[71]	4.0
GNAS/(guanine nucleotide binding protein, alpha stimulating) complex locus	D		NC		(D) DBP ST Blood[70]	(Transgenic) Abnormal response to new environment				4.0
GRN/granulin		I	NC			(Transgenic) Increased aggression		(D) Chronic stress[48]		4.0
HSP90AA1/heat shock protein 90, alpha (cytosolic), class A member 1	I	I	NC	(D) DBP ST PFC[70]						4.0
KLK1/kallikrein 1	I	I	NC	(I) Anxiety[102]						4.0
KLK1B27/kallikrein 1-related peptidase b27	I	I	NC		(D) DBP ST Blood[70]					4.0
LBR/lamin B receptor		I	NC			(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	1q42.12 (Linkage) Autism/OCD[103] PD[75]	4.0
MCL1/myeloid cell leukemia sequence 1		I	NC			(QTL) Abnormal emotion/affect behavior		(D) High lonely individuals (social epidemiological risk factor)[107]	1q21.3 (Linkage) anxiety[122]	4.0
NUCKS1/nuclear casein kinase and cyclin-dependent kinase substrate 1	D		NC	(D) DBP ST PFC (I) DBP ST AMY[70]		(QTL) Abnormal emotion/affect behavior			1q32.1 (Linkage) PD[71]	4.0
PCNA/proliferating cell nuclear antigen	I	I	NC					(D) Stress[83]		4.0
PDCD6/programmed cell death 6		I	NC			(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	3p22.3 (Linkage) Anxiety/PD[82]	4.0

Table 2 Continued

BLOOD (BLD) Gene symbol/name	*BLD YH*	*BLD DZ*	*BLD Co-TX*	*Animal models brain evidence*	*Animal models blood evidence*	*Animal genetic (QTL/transgenic) evidence*	*Human brain evidence*	*Human blood evidence*	*Human genetic (linkage/ association) evidence*	*CFG score*
PPBP/pro-platelet basic protein	I	D	NC			(QTL) Abnormal emotion/affect behavior			4q13.3 (Linkage) Anxiety[10]	4.0
PRDX1/peroxiredoxin 1	I	I				(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	1p34.1 (Linkage) Neuroticism[74]	4.0
RPL14/ribosomal protein L14		I	NC	(D) Anxiety[129]				(D) PD[73]		4.0
RPL3/ribosomal protein L3		I	NC	(D) Stress[97]		(QTL) Abnormal emotion/affect behavior			22q13.1 (Linkage) PD[113] Harm avoidance (anxiety proneness)[130]	4.0
RPL30/ribosomal protein L30	I	I	NC	(D) Stress[77,97]						4.0
RPS27/ribosomal protein S27	I	I		(D) Anxiety[129]		(QTL) Abnormal emotion/affect behavior			1q21.3 (Linkage) Anxiety[122]	4.0
RPS3/ribosomal protein S3	I	I	NC	(D) Stress[97]						4.0
SNX17/sorting nexin 17	I	I	NC					(D) Chronic stress[48]		4.0
TXNIP/thioredoxin-interacting protein		I	NC			(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	1q21.1 (Linkage) Anxiety[122]	4.0
ZFP36L2/zinc-finger protein 36, C3H type-like 2		I	NC			(QTL) Abnormal emotion/affect behavior		(D) Chronic stress[48]	2p21 (Linkage) PD[89]	4.0

Abbreviations: AMY, amygdala; BLD, blood; CFG, convergent functional genomics; Co-TX, co-treatment; D, decreased; DBP, D-box binding protein; DZ, diazepam; I, increased; HIP, hippocampus; NC, no change; OCD, obsessive compulsive disorder; PFC, prefrontal cortex; PD, panic disorder; PPI, prepulse inhibition; PTSD, post-traumatic stress disorder; QTL, quantitative trait loci; ST, stressed; YH, yohimbine.
Top candidate genes (CFG score of 4.0 points and above) from PFC ($n=16$), AMY ($n=19$), HIP ($n=45$) and BLD ($n=41$).

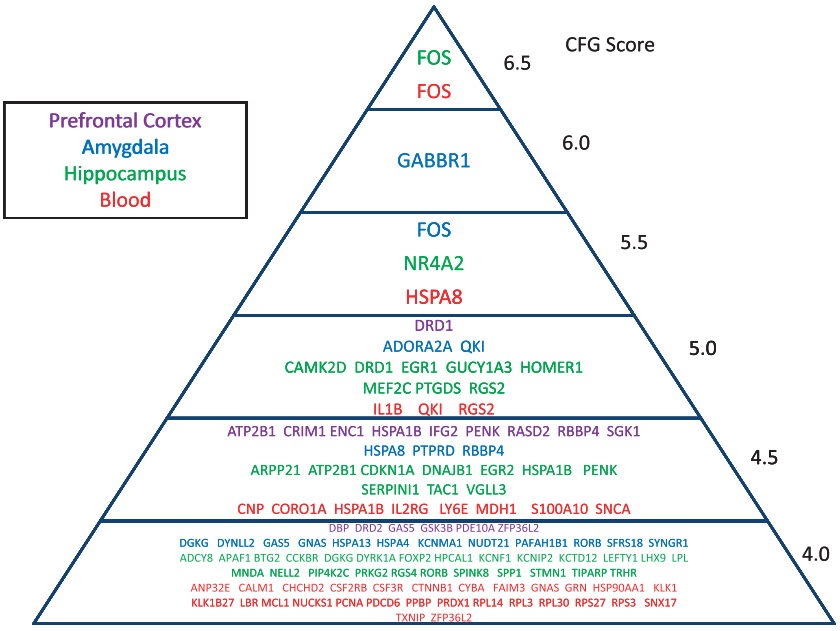


Figure 3 Top candidate genes for anxiety.

disorders may thus be more important than previously appreciated, consistent with recent work in the field.[32,39]

Top candidate genes. Our analysis identified and prioritized a number of top candidate genes (Figure 3 and Table 2), some well-known for involvement in anxiety, some less well known, such as *FOS*, *GABBR1*, *NR4A2*, *DRD1*, *ADORA2A*, *QKI*, *RGS2*, *PTGDS*, *DYNLL2* and *CCKBR*. FOS (FBJ murine osteosarcoma viral oncogene homolog) is an oncogene as well as an immediate early response gene. It is a transcription factor involved in cellular reactivity to external signals. In our studies, it is also a top brain–blood biomarker for anxiety, concordantly changed in the AMY, HIP and blood. Interestingly, there is previous evidence of increase in expression of FOS in blood from PTSD patients.[40] GABBR1 (gamma-aminobutyric acid (GABA) B receptor, 1) has a critical role in the fine-tuning of inhibitory synaptic transmission mediated by GABA. Our work provides evidence for its involvement in the AMY in anxiety (Table 2). Like FOS, it is also changed (decreased) in expression in blood from PTSD patients.[40] GABBR1 has previous evidence suggestive for genetic association with OCD[41] and with schizophrenia.[42] NR4A2 (nuclear receptor subfamily 4, group A, member 2) is a steroid receptor family member, as well as immediate early response gene. It is a transcription factor involved in cellular reactivity to external signals, with a role in dopaminergic neuron development. Our work provides evidence for its involvement in the HIP in anxiety (Table 2). NR4A2 has previous evidence suggestive for genetic mutations[43] and brain expression changes[44] in schizophrenia and bipolar disorder. DRD1 (dopamine receptor 1), for which our work provides evidence for its involvement in the PFC and HIP in anxiety (Table 2), has previous evidence suggestive for genetic association in panic disorder.[45] ADORA2A (adenosine A2a receptor), is a receptor for adenosine. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase. Our work provides evidence for the involvement of ADORA2A in the AMY in anxiety (Table 2). There is previous evidence suggestive for genetic association in panic disorder.[46,47] Notably, with the exception of FOS, all the above discussed top candidate genes for anxiety have also been previously identified by our CFG work as being among top candidate genes for schizophrenia[29] (Table 6, Supplementary Figure S1). QKI (quaking homolog, KH domain RNA binding), a RNA-binding protein, has a central role in myelination. In our studies, it is also a top brain–blood biomarker for anxiety, concordantly changed in the AMY and blood. Interestingly, there is previous evidence of increase in expression of QKI in blood from humans subjected to chronic stress.[48] Finally, among our top candidate genes are *RGS2*, *DYNLL2*, *PTGDS* and *CCKBR*, all of which have previous human genetic association evidence for anxiety disorders and thus serve as a *de facto* positive control for our pharmacogenomic approach. Of note, *PTGDS* and *CCKBR* are also top candidate genes for schizophrenia in our previous work[29] (Table 6, Supplementary Figure S1).

In addition, we have looked at what genes were changed in expression in all three brain regions studied (Table 3), on the premise they are more likely to be involved in the core biology of anxiety. Notably, EGR2 (early growth response 2) and SGK1 (serum/glucocorticoid-regulated kinase 1), which are involved in cellular reactivity to external signals and stress, have high CFG scores (i.e. multiple converging lines of evidence) for involvement in anxiety disorders.

Biomarkers. Genes that are changed in expression in one of the key brain regions studied and in blood are candidate blood biomarkers.[22] We used a narrow interpretation of what can constitute a candidate blood biomarker (Table 4), i.e. the

Table 3 Genes changed in expression in all three brain regions

Gene	*PFC YH*	*PFC DZ*	*PFC Co-TX*	*AMY YH*	*AMY DZ*	*AMY Co-TX*	*HIP YH*	*HIP DZ*	*HIP Co-TX*	*CFG score*
EGR2 (early growth response 2)		I	NC	I		NC	I	I	NC	4.5
SGK1 (serum/glucocorticoid regulated kinase 1)	I	I	NC	I	I		I			4.5
MEG3 (maternally expressed 3	D	D		D	D		I	I		3.5
FABP7 (fatty acid-binding protein 7, brain)	I			I			I			3.0
TTR (transthyretin)		D		D		NC	I	I		3.0
IGFBP2 (insulin-like growth factor-binding protein 2)		D	NC		D	NC	I		NC	3.0
NPAS4 (neuronal PAS domain protein 4)	D			D				I		3.0
ERDR1 (erythroid differentiation regulator 1)	D			D			D			2.0

Abbreviations: AMY, amygdala; CFG, convergent functional genomics; Co-TX, co-treatment; D, decreased; DZ, diazepam; HIP, hippocampus; I, increased; NC, no change; PFC, prefrontal cortex; YH, yohimbine.

Table 4 Top candidate brain–blood biomarkers for anxiety

PFC-blood	*PFC YH*	*PFC DZ*	*PFC Co-TX*	*Blood YH*	*Blood DZ*	*Blood Co-TX*	*Animal models brain evidence*	*Animal models genetic evidence*	*Human blood evidence*	*Human genetic evidence*	*CFG Score*
HSPA1B (heat shock protein 1B)		I	NC		I	NC	(I) DBP ST PFC[42]		(I) Chronic stress[46]	6p21.33 (Linkage) Neuroticism[50] PD[51]	4.5
HIST1H1C (histone cluster 1, H1c)	I			I		NC					2.0
AMY-blood	***AMY YH***	***AMY DZ***	***AMY Co-TX***	***Blood YH***	***Blood DZ***	***Blood Co-TX***	***Animal models brain evidence***	***Animal models genetic evidence***	***Human blood evidence***	***Human genetic evidence***	***CFG score***
FOS (FBJ osteosarcoma oncogene)		I	NC	D	I	NC	(I) DBP ST AMY[42] (I) Anxiety[63] (I) Stress)[53]	(Transgenic) Decreased anxiety-related response	(I) PTSD[64] (I) Stress[54] Stress[65]	(I)14q24.3 (Linkage) OCD[66]	6.5
QKI (quaking homolog, KH domain RNA-binding (mouse))	I		NC	I		NC	(I) DBP ST AMY[42]	(Transgenic) Abnormal response to novel object	(D) Chronic stress[46]		5.0
A130040 M12Rik RIKEN cDNA	I		NC	I	I	NC					3.0
TSC22D3 (TSC22 domain family, member 3)	I	I			I	NC					2.0
HIP-Blood	***HIP YH***	***HIP DZ***	***HIP Co-TX***	***Blood YH***	***Blood DZ***	***Blood Co-TX***	***Animal models brain evidence***	***Animal models genetic evidence***	***Human blood evidence***	***Human genetic evidence***	***CFG score***
FOS (FBJ osteosarcoma oncogene)	I	I	NC	D	I	NC	(I) DBP ST AMY[42] (I) Anxiety[63] (I) Stress[53]	(Transgenic) Decreased anxiety-related response	(I) PTSD[64] (I) Stress[54] Stress[65]	(I)14q24.3 (Linkage) OCD[66]	6.5
DNAJB1 (DnaJ (Hsp40) homolog, subfamily B, member 1)	I	I	NC		I	NC	(D) Stress[89]	(QTL) Abnormal emotion/ affect behavior			4.5

Abbreviations: AMY, amygdala; CFG, convergent functional genomics; Co-TX, co-treatment; D, decreased; DZ, diazepam; HIP, hippocampus; I, increased; NC, no change; PFC, prefrontal cortex; QTL, quantitative trait loci; YH, yohimbine.
Co-directional brain–blood gene expression changes.

change in gene expression in brain and blood has to be co-directional, inside the same drug treatment arm. FOS, QKI, HSPA1B and DNAJB1 are the top candidate biomarkers under this definition. There is more overlap between brain and blood if co-directionality of expression is not a criterion (Supplementary Table S1), as different tissues (and brain regions) can show different directions of gene expression changes. Moreover, there may be an even more significant overlap between brain and blood at a biological pathway level (Table 5 and Figure 4), where the same top pathways, if not necessarily the same genes, show alterations. Notably the glucocorticoid receptor signaling pathway and the CCR5 signaling pathway are altered in anxiety in both AMY and blood. In the end, panels of biomarkers and pathways need to be clinically validated, i.e. show predictive ability for anxiety state or response to treatment in independent human studies.

Table 5 Biological pathway analyses of top candidate genes

Top Canonical Pathways	P-***value***	***Ratio***
PFC (n = 16 genes)		
cAMP-mediated signaling	1.33E−03	3/217 (0.014)
Huntington's disease signaling	1.65E−03	3/246 (0.012)
Dopamine receptor signaling	3.69E−03	2/93 (0.022)
Glioma signaling	5.11E−03	2/116 (0.017)
PTEN signaling	5.6E−03	2/123 (0.016)
AMY (n = 19 genes)		
Aldosterone signaling in epithelial cells	3.51E−05	4/172 (0.023)
Protein ubiquitination pathway	2.15E−04	4/274 (0.015)
cAMP-mediated signaling	1.85E−03	3/217 (0.014)
Glucocorticoid receptor signaling	3.21E−03	3/284 (0.011)
CCR5 signaling in macrophages	3.37E−03	2/95 (0.021)
HIP (n = 45 genes)		
cAMP-mediated signaling	2.39E−04	5/217 (0.023)
Corticotropin releasing hormone signaling	2.89E−04	4/137 (0.029)
GNRH signaling	3.58E−04	4/145 (0.028)
G-protein coupled receptor signaling	3.83E−04	7/531 (0.013)
Antiproliferative role of somatostatin receptor 2	1.08E−03	3/81 (0.037)
Blood (n = 41 genes)		
Glucocorticoid receptor signaling	5.21E−04	5/284 (0.018)
CCR5 signaling in macrophages	9.33E−04	3/95 (0.032)
PPAR signaling	1.84E−03	3/106 (0.028)
Role of osteoblasts, osteoclasts and chondrocytes in rheumatoid arthritis	2.67E−03	4/243 (0.016)
Huntington's disease signaling	2.88E−03	4/246 (0.016)

Abbreviations: AMY, amygdala; CFG, convergent functional genomics; HIP, hippocampus; PFC, prefrontal cortex.
Ingenuity Pathway Analyses of top candidate genes (CFG score of 4.0 and up).

Pathways. First, we carried out biological pathway analyses on all the genes that were changed in expression in our pharmacogenomic model, without any CFG prioritization (Supplementary Table S2). This may give a view of pathways involved in anxiety in the brain, but probably includes other pleiotropic effects of the drugs used.

Next, we carried out pathway analyses on the top candidate genes prioritized by CFG (CFG score of 4.0 and above) (Table 5). The resulting pathways are likely more specific to the core illness phenomenology, and less pleiotropic. Among these top biological pathways altered in anxiety, cAMP is changed in common in all three brain regions studied (Figure 4). cAMP signaling is fundamental to cellular reactivity to external signals. Previous evidence has been suggestive of a role for cAMP signaling pathways in anxiety disorders,[49–51] but our work is the first to identify it as a core mechanism for anxiety across different brain regions.

We also identified biological pathways involved in anxiety specific to the different brain regions we studied. In the PFC, after cAMP signaling, the top pathway is Huntington's disease signaling. This pathway is also a top pathway altered in the blood in our analyses, suggesting its potential as a biomarker repository (Figure 4). In the AMY, the top pathway is aldosterone signaling. Previous work in animal models has suggested a role for the mineralocorticoid pathway in anxiety and stress response.[52] Glucocorticoid receptor signaling and CCR5 signaling are other top pathways in the AMY, as well as in blood (Figure 4). In the HIP, after cAMP signaling, the top pathway is corticotropin-releasing hormone signaling. This pathway is well established in anxiety and stress

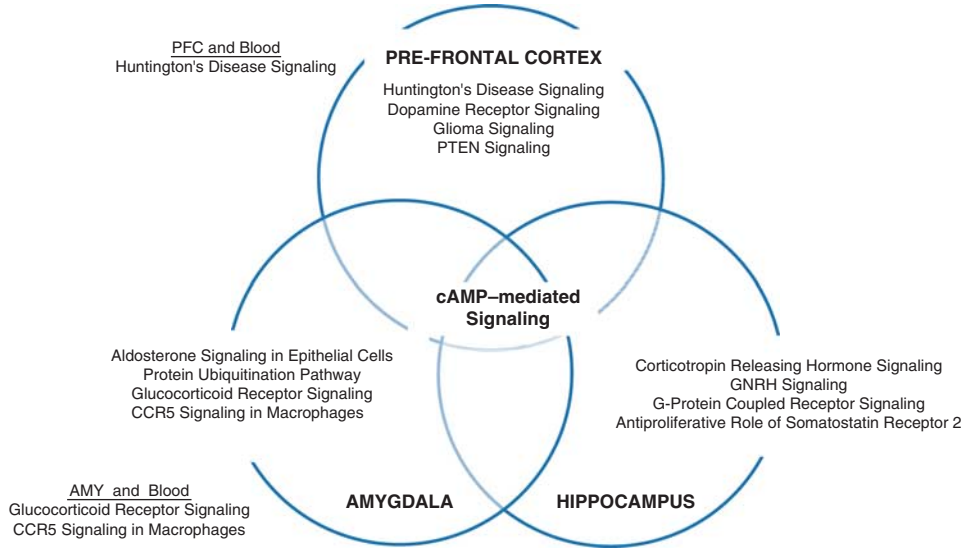


Figure 4 Top biological pathways for anxiety in different brain regions. Overlap between brain regions, and with the blood.

response,[53,54] and serves as a reassuring positive control for our own work and analyses.

Discussion

We have used a comprehensive, CFG approach for identifying high probability candidate genes, pathways and mechanisms for anxiety and related disorder, by the integration in a Bayesian fashion of multiple independent converging lines of evidence. This mapping of the genomic landscape of anxiety disorders completes our triad of first-pass mapping efforts of major psychiatric disorders domains—bipolar disorder,[25,26,55] schizophrenia,[29] and now anxiety disorders.

Our convergent approach emphasizes gene expression evidence more than genetic evidence, i.e. more of the scored lines of evidence come from gene expression studies than from genetic studies (Figure 1). Gene expression studies are arguably a better way to understand biology than genetic studies. After all, gene expression is the result of integration of the effects of many genetic polymorphisms, epigenetic changes and environmental effects, whereas genetics looks too early in this chain of events, and in a narrow fashion. Biologically important genes can thus be identified and studied in action at a gene expression level, whereas at a genetic level the complexity and heterogeneity of genetic polymorphisms precludes easy identification and gives no indication of their actual biological activity. The advantage of gene expression studies over genetic studies, including sequencing, may be magnified by evolutionary considerations of increased genetic heterogeneity in highly biologically active and environmentally reactive genes, such as brain and immune system genes, as a way of permitting adaptation to the environment.[23] Moreover, as per our earlier formulation that 'genes that change together (may) act together',[24] the co-expression data sets we have generated in various brain regions offer testable hypotheses for transcriptional co-regulation, and for epistatic interactions among the corresponding loci.[56]

Limitations and confounds. An acute treatment model like the one we are using is not necessarily inductive to assessing the long-term changes associated with anxiety, such as functional and structural changes apparent on imaging. Although we have no direct way of knowing if some of the genes we captured with our screen are involved or not in setting in motion such long-term changes, it is to be noted that some of these gene changes have also been reported in genetic studies of anxiety and anxiety-related disorders. Moreover, we have candidate genes in our data set with roles in brain infrastructure, including myelination (Table 2). More chronic treatments should, nevertheless, be pursued to verify and expand the findings presented in this paper.

Different combinations of anxiogenic and anxiolytic agents could be used in a comprehensive functional pharmacogenomic approach such as the one we have described. They could conceivably lead to different results, which would be of interest and welcome, since it is unlikely we are capturing with our model the full spectrum of gene expression changes involved in anxiety. However, if those drug combinations indeed mimic and modulate the same core phenomenology, the Venn diagrams of the overlap between different drug treatments will be of high interest in terms of identifying the key molecular players involved in the effects, as opposed to those involved in the (very different) side-effects of the individual drugs.

It is to be noted that our experimental approach for detecting gene expression changes relies on a single methodology, Affymetrix GeneChip oligonucleotide microarrays. It is possible that some of the gene expression changes detected from a single biological experiment, with a one-time assay with this technology, are biological or technical artifacts. With that in mind, we have designed our experiments to minimize the likelihood of having false positives, even at the expense of having false negatives. Working with an isogenic mouse strain affords us an ideal control baseline of saline vehicle injected animals for our drug-injected animals. We performed three independent *de novo* biological experiments, at different times, with different batches of mice (Figure 1b). We have pooled material from three mice in each experiment, and carried out microarray studies. The pooling process introduces a built in averaging of signal. We used a Venn diagram approach and only considered the genes that were reproducibly changed in the same direction in at least two out of three independent experiments. This overall design is geared to factor out both biological and technical variability. It is to be noted that the concordance between reproducible microarray experiments using the latest generations of oligonucleotide microarrays and other methodologies such as quantitative PCR, with their own attendant technical limitations, is estimated to be over 90%.[57] Moreover, our CFG approach, as described above, is predicated on the existence of multiple internal and external cross-validators for each gene that is reproducibly changed in expression (Figure 1). These cross-validators are derived from independent gene expression or genetic experiments.

Conclusions and future directions. The results presented in this paper have a series of direct implications. First, in terms of pharmacotherapy and drug development, some of the candidate genes in our data set encode for proteins that are modulated by existing pharmacological agents (Supplementary Table S4), which may suggest future avenues for rational polypharmacy using currently available agents. Notably, existing drugs approved for other indications, such as dopaminergic agents, ion channel blockers, baclofen, nitrates, lipid modulators and disulfiram (Antabuse) are potential augmentation options for existing first-line anxiolytics and merit careful exploration as such. Some of the top anxiety candidate genes (*FOS*, *PTGDS*, *HOMER1*, *NR4A2*, *GSK3B* and *LPL*) are also modulated by the omega-3 fatty acid DHA in recent animal model studies carried by us (Le-Niculescu *et al.*, *Transl Psychiatry* (2011) 1, e4, doi:10.1038/tp.2011.1), providing a potential non-pharmacological alternative for treatment. Our data sets of the effects of yohimbine and diazepam on gene expression in different key brain regions (Table 2) may be used as a source of new targets for drug development. The candidate biomarkers identified by us may, upon future validation, aid with drug development, monitoring response to treatment

Table 6 Gene Overlap Across Psychiatric Disorders: a CFG view

Anxiety	***Bipolar***[25,26,55]	***Schizophrenia***[29]	***Alcohol***[31]
ADORA2A		ADORA2A	
BTG2			BTG2
CCKBR		CCKBR	
DBP	DBP[55]		
DRD1		DRD1	
DRD2		DRD2	
FOXP2		FOXP2	
GABBR1		GABBR1	
GNAS			GNAS
GSK3B	GSK3B[26]		
LPL		LPL	
MEF2C	MEF2C[25]		
NR4A2		NR4A2	
PDE10A	**PDE10A**[26]	**PDE10A**	
PENK	PENK[25]		
PTGDS		PTGDS	
RGS4		RGS4	
RORB	RORB[26]		
TAC1	**TAC1**	**TAC1**	

Abbreviation: CFG, convergent functional genomics.
Top anxiety CFG candidate genes are also top CFG candidate genes for other major psychiatric disorders based on our previous studies.[25,26,31,55]

Table 7 Disease analyses for top candidate genes

GeneGo disease analyses ***Disease***	P-***value***	***Ratio***
PFC (n = *16 genes)*		
Depressive disorder, major	3.101e−14	10/133
Depressive disorder	1.034e−12	10/188
Mood disorders	3.462e−12	12/410
Parkinson disease	2.453e−11	11/361
Parkinsonian disorders	5.961e−11	11/392
AMY (n = *19 genes)*		
Mood disorders	5.074e−7	8/410
Friedreich ataxia	5.087e−7	3/9
Agoraphobia	7.258e−7	3/10
Fibrosis	1.546e−6	8/475
Genetic syndromes sometimes associated with diabetes	3.362e−6	3/16
HIP (n = *45 genes)*		
Mental disorders	7.094e−15	41/2290
Psychiatry and psychology	1.303e−14	41/2329
Schizophrenia	5.021e−14	26/838
Schizophrenia and disorders with psychotic features	5.618e−14	26/842
Cough	1.975e−12	7/17
Blood (n = *41 genes)*		
Schizophrenia and disorders with psychotic features	4.592e−9	16/842
Wounds and injuries	4.708e−9	17/977
Urogenital neoplasms	1.930e−8	25/2531
Genital diseases, male	3.695e−8	24/2391
Schizophrenia	3.736e−8	15/838

Abbreviations: AMY, amygdala; CFG, convergent functional genomics; HIP, hippocampus; PFC, prefrontal cortex.
Disease grouping analysis of top candidate genes (CFG score of 4.0 and up). GeneGo analyses.

and early clinical intervention. Heterogeneity is possible, indeed likely, in individual human subjects—a fertile direction for future studies.[26,28,30] Targeting key pathways identified by us (Figure 4) may provide broader options than targeting individual genes, for both drug development and peripheral blood readouts.

Second, despite using lines of evidence for our CFG approach that have to do only with anxiety disorders, the list of genes identified has a notable overlap with other psychiatric disorders, and with medical disorders (Tables 5 and 7, Supplementary Tables S2 and S3). This is a topic of major interest and debate in the field.[58,59] We demonstrate an overlap between top candidate genes for anxiety and candidate genes for schizophrenia and bipolar disorder, as well as alcoholism previously identified by us through CFG (Table 6 and Supplementary Figure S1), thus providing a possible molecular basis for the frequently observed clinical co-morbidity and interdependence between anxiety and those other major psychiatric disorders. Notably, PDE10A and TAC1 are at the overlap of all three major psychiatric domains, and may be of major interest for drug development.[60–62] Among our top candidate genes for anxiety are *DBP* and *RORB*, circadian clock genes previously identified by us as candidate genes for bipolar disorder[27,55,63] (Table 6 and Supplementary Figure S1). In addition to mood symptoms, we had previously demonstrated that DBP knock-out mice exhibit increased reactivity to stress, as well as increased alcohol consumption.[27] *NPAS4*, another circadian gene in our anxiety dataset, is changed in expression in all three brain regions studied (Table 3). NPAS4 is a transcription factor that acts as a heterodimer partner for *ARNTL*, another top candidate gene for bipolar disorder identified by our previous work.[25,26,64] The involvement of circadian genes in anxiety may underlie anxiety effects on sleep, diurnal variations in anxiety (for example, higher at night), and cycling in levels of anxiety symptoms in some patients—similar too, driven by or driving mood symptoms (cycloanxiety vs cyclothymia).[6] Another top candidate gene at the overlap of bipolar disorder and anxiety is *PENK* (preproenkephalin). Our work provides evidence for the involvement of PENK in the PFC and HIP in anxiety (Table 2). Endogenous opiates may signal that the environment is favorable, improving mood and decreasing anxiety. As such, exogenous opiate drugs may be effective for treatment, but highly addictive. Unexpectedly, there is a major overlap between schizophrenia and anxiety, both at a top candidate genes level (Supplementary Figure S1 and Table 6) and at a pathway analyses level (Table 7 and Supplementary Table S3). Clinically, while there are some reports of co-morbidity between schizophrenia and anxiety,[65–67] it is an area that has possibly been under-appreciated and understudied. Based on our work and the body of evidence in the field, we propose that a new diagnostic category of 'schizoanxiety disorder' may have heuristic value and pragmatic clinical utility, similar to schizoaffective disorder.

Third, the mechanistic understanding and model for anxiety that emerges out of the candidate gene identified and the analyses of biological pathways involved points to signal transduction and reactivity to signals from the external environment and internal milieu (Figure 5). Notably, pathways involved in cellular stress and heat shock response (involving HSPA1B, HSPA8, HSPA4, HSPA13) seem to have been recruited by evolution for higher whole-body and mental functions[68] such as anxiety. The cybernetic-like simplicity of the model should not overshadow the important fact that it is

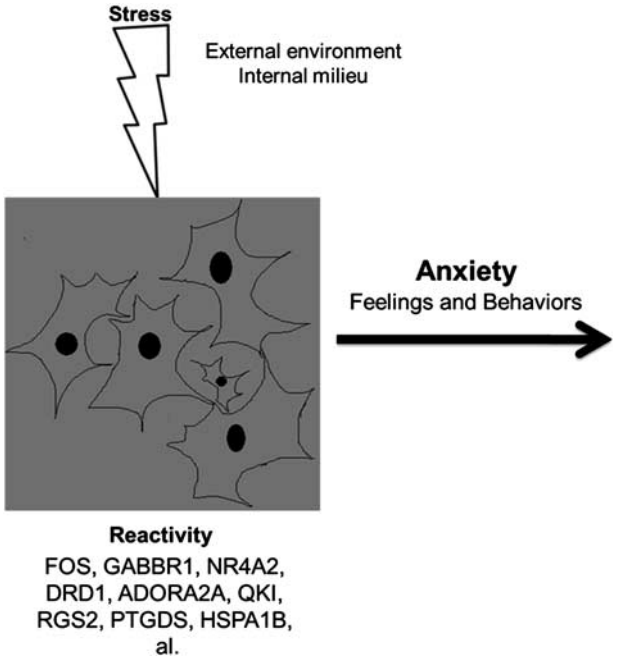


Figure 5 Anxiety disorders: reactivity to the environment.

the result of the empirical coalescence of data in a non-hypothesis driven, discovery type approach. The implications for understanding the pathophysiology and treatment of anxiety and related disorders are profound. One needs to correct cellular, brain and whole organism reactivity to the environment in the treatment of these disorders. It is a place where psychopharmacology, management of medical problems, cognitive–behavioral therapy and social integration can and should go hand in hand.

In conclusion, we propose that our comprehensive CFG approach is a useful starting point in helping unravel the complex genetic code and neurobiology of anxiety and related disorders, and generates a series of leads for both future research and clinical practice.

Conflict of interest

The authors declare no conflict of interest.

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