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A large-scale candidate gene analysis of mood disorders: evidence of neurotrophic tyrosine kinase receptor and opioid receptor signaling dysfunction

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Abstract

Background—Despite proven heritability, little is known about the genetic architecture of mood disorders. Although a number of family and case–control studies have examined the genetics of mood disorders, none have carried out joint linkage-association studies and sought to validate the results with gene expression analyses in an independent cohort.

Methods—We present findings from a large candidate gene study that combines linkage and association analyses using families and singletons, providing a systematic candidate gene investigation of mood disorder. For this study, 876 individuals were recruited, including 83 families with 313 individuals and 563 singletons. This large-scale candidate gene analysis included 130 candidate genes implicated in addictive and other psychiatric disorders. These data showed significant genetic associations for 28 of these candidate genes, although none remained significant after correction for multiple testing. To evaluate the functional significance of these 28 candidate genes in mood disorders, we examined the transcriptional profiles of these genes within the dorsolateral prefrontal cortex and anterior cingulate for 21 cases with mood disorders and 25 nonpsychiatric controls, and carried out a pathway analysis to identify points of high connectivity suggestive of particular molecular pathways that may be dysregulated.

Results—Two primary gene candidates were supported by the linkage-association, gene expression profiling, and network analysis: neurotrophic tyrosine kinase receptor, type 2 (*NTRK2*), and the opioid receptor, $\kappa 1$ (*OPRK1*).

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Conflicts of interest

There are no conflicts of interest.

Conclusion—This study supports a role for *NTRK2* and *OPRK1* signaling in the pathophysiology of mood disorder. The unique approach incorporating evidence from multiple experimental and computational modalities enhances confidence in these findings.

Keywords

linkage and association; mood disorders; neurotrophic tyrosine kinase receptor; opioid receptor; type 2; $\kappa 1$

Introduction

Mood disorders affect ~10 million Americans annually, and are a major cause of disability in the USA. They are a group of multifactorial diseases with both genetic and environmental etiological factors. Twin studies indicate an estimated heritability of 40%, with a two-fold to three-fold increased risk for the first-degree relatives of probands (Sullivan *et al.*, 2000). Although a number of studies have investigated the genetic architecture of depression, the genetic etiology of mood and depressive disorders remains poorly understood. Family-based linkage studies have identified a number of chromosomal regions (Shyn and Hamilton, 2010) that include brain-related candidate genes such as neurotrophic tyrosine kinase receptor, type 3 (*NTRK3*), and solute carrier family 6 [neurotransmitter transporter, γ -aminobutyric acid (*GABA*)], member 4 (*SLC6A4*) (Middeldorp *et al.*, 2009). However, these findings have yet to be replicated. Case-control studies of candidate genes in depression have not yielded robust findings (Shyn and Hamilton, 2010). A recent meta-analysis of mood disorders suggests an association with chromosome 3 (McMahon *et al.*, 2010), and another large meta-analysis (Lopez-Leon *et al.*, 2008) did report an association with three genes: apolipoprotein E, guanine nucleotide binding protein (G protein), $\beta 3$ (*GNB3*), and *SLC6A4*. A recent genome-wide study by Shyn *et al.* (2011) did not identify a single locus with genome-wide significance (Shyn *et al.*, 2011), but reported suggestive findings for single nucleotide polymorphisms (SNPs) within the ATPase, H^+ transporting, lysosomal V1 subunit B2 (*ATP6V1B2*), Sp4 transcription factor (*SP4*), and glutamate receptor, metabotropic 7 (*GRM7*) genes. Other findings from genome-wide associations studies have identified the genes *PCLO* and *HOMER1* in depression (Sullivan *et al.*, 2009; Rietschel *et al.*, 2010), the genes *NCAN* and *ANK3* in bipolar disorder (Ferreira *et al.*, 2008; Cichon *et al.*, 2011), and the gene *TMEM132D* in panic disorder and unipolar depression (Erhardt *et al.*, 2011).

In this study, we examine the genetic basis of mood disorders by using a joint family and case-control strategy that combines two study approaches in one analytical framework. We carried out a large-scale candidate gene analysis with 130 candidate genes that have been implicated previously in addictive and other psychiatric disorders. Significant comorbidity exists between substance abuse and mood disorder (Regier *et al.*, 1990), and numerous candidate genes have been found to be implicated in both mood disorder and substance abuse (Edwards *et al.*, 2012). For instance, the opioid receptor, $\mu 1$ (*OPRM1*), has been associated with antidepressant efficacy (Garriock *et al.*, 2010) and depressive symptoms in alcohol-dependent individuals (Kertes *et al.*, 2010), opioid receptor, $\delta 1$ (*OPRD1*) knockout mice show a depressive phenotype (Filliol *et al.*, 2000), and stimulation of opioid receptors has an antidepressant effect (Broom *et al.*, 2002; Jutkiewicz *et al.*, 2004; Saitoh *et al.*, 2004). Such findings involving abnormalities of reward mechanisms in mood disorders suggest that other components of the molecular pathways implicated in addictive disorders may also be dysregulated in mood disorders. Over 50% of the addictive disorder candidate genes evaluated in the linkage-association segment of this study have also been implicated previously in mood disorders (Zubenko *et al.*, 2003; Wong *et al.*, 2008; Lopez-Leon *et al.*, 2008; Dong *et al.*, 2009; Bosker *et al.*, 2011; Kao *et al.*, 2011). The objective was both to

confirm previously identified and to identify novel genetic associations between addictive disorder candidate genes and mood disorders, thus further unraveling the interrelationship between these highly comorbid disorders.

We adopted a joint family and case-control approach to maximize the power of the study, combining familial cases of mood disorders, as well as population-based mood disorder cases and controls. For this study, 876 individuals were recruited, including 83 families with 313 individuals and 563 singletons. Candidate genes that showed suggestive linkage-association were analyzed in a pathway analysis to identify molecular pathways that may be dysregulated. We then examined gene expression levels for these candidate genes as well as novel genes identified through the pathway analysis, within the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate (AC) in post-mortem samples from an independent set of patients with 21 mood disorder and 25 controls.

Methods

Samples and participants

This study combines family and case-control study designs, and as such, we used a collection of families and singletons. The study sample comprised 876 individuals, including 83 families with 313 individuals, and 563 singletons. Among the families, a total of 143 (46.0%) individuals had a mood disorder and were considered as affected, 79 (25.2%) individuals had no psychiatric disorder and were considered as unaffected, and the remaining individuals in the families who did not fulfill either the affected or the unaffected diagnostic criteria were assigned an unknown phenotypic status for the purpose of data analysis. Similarly, the singletons for the case-control part of the analysis comprised 382 affected and 179 unaffected individuals. The individuals designated as affected encompassed a broad diagnostic phenotype of mood disorders, including those with diagnoses of major depressive disorder, dysthymia, or bipolar disorder, while excluding patients with schizophrenia or schizoaffective disorders. The median age of onset for the mood disorder sample was 21 years (interquartile range: 16–35 years), and the median number of depressive episodes was three episodes (range: 1–6). We used a broad diagnostic model for the present study to account for the continuum of behavioral and diagnostic symptoms that manifest in mood disorders. Major depression, dysthymia, and bipolar disorder share behavioral symptoms, most obviously depressed mood, and hence, examination of such a broad diagnostic model might lead to the identification of common genetic pathways contributing toward depressed mood.

The familial samples were collected as part of the familial pathways to the early-onset suicide attempts project described previously (Brent *et al.*, 2002; 2003; 2004), where blood samples were collected from probands with major depression and offspring. The singleton samples were obtained as part of an ongoing study of suicidal behavior in mood disorders (Oquendo *et al.*, 2004; Galfalvy *et al.*, 2006). All patients had been referred for evaluation of depressive episodes to a university clinic or had responded to advertisements for patients with a major depressive episode or healthy volunteers. Patients were assessed by research psychiatrists and clinical research psychologists, and diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. criteria for Axis I diagnoses using the structured clinical interview SCID-I/P or SCID-NP (First and Pincus, 2002). Of the 525 affected cases with mood disorders, 414 had a diagnosis of major depressive disorder/dysthymia and 111 had a diagnosis of bipolar disorder, where 111 of these cases with mood disorders also had comorbid alcohol dependence and 40 had other substance use disorders (Supplementary Figure S1, <http://links.lww.com/PG/A67>). The familial samples included 41.7% men and 58.3% women, mean age 33.0 years. Singleton individuals included 45.3% men and 54.7% women, mean age 40.7 years.

The racial and ethnic compositions of the samples were determined on the basis of self-report and were limited to Caucasian ancestry to reduce the effects of population stratification. The exclusion criteria for the patient group included the presence of persistent psychotic symptoms or psychotic disorder diagnosis, a history of severe head trauma, or the presence of mental retardation or any cognitive impairment that might interfere with the completion of clinical assessments. The study was approved by the applicable Institutional Review Boards, and all participants provided written informed consent.

Genotyping

Samples were genotyped using an beadchip (Illumina, San Diego, California, USA) microarray containing a total of 1350 SNPs within 130 candidate genes implicated in addiction and alcoholism (Hodgkinson *et al.*, 2008). DNA samples were isolated as described previously from buffy coat fraction and from buccal mucosa cheek swabs (Higuchi, 1992; Huang *et al.*, 2003) (BuccalAmp DNA Extraction Kit; Epicentre, Madison, Wisconsin, USA). Genotyping was performed using the Illumina Gold-enGate genotyping protocols on 96-well format Sentrix arrays. Five hundred nanograms of sample DNA was used per assay. All pre-PCR processing was performed using a TECAN liquid handling robot running Illumina protocols. Arrays were imaged using an Illumina Beadstation GX500 and the data were analyzed using GenCall v6.2.0.4 and GTS Reports software v5.1.2.0 (Illumina). All arrays included DNA samples from both affected and unaffected individuals. Genotype data quality control and filtering was performed as described previously (Hodgkinson *et al.*, 2008). Briefly, genotypes with low GenCall scores (< 0.25) were considered as undetermined. The GenCall score is a value between 0 and 1, yielding a confidence score for that genotype call (the higher the score, the higher the confidence in the call), and is derived from the tightness of the clusters for a given locus and the position of the sample relative to its cluster. The cluster plots for all SNPs were examined individually and where there was insufficient separation of the three genotype clusters, the SNP was not included in the analyses. Loci with a call rate more than 90% were included. In addition, SNPs were excluded from analysis if the minor allele was detected in fewer than 5% of the samples. Of the total 1350 loci genotyped, 1045 (77%) passed these quality control criteria and were subsequently used in downstream analyses. Furthermore, the genotyping call rates did not vary by affection status, or the source and method of DNA preparation. Finally, all genotypes from the family data were examined for mendelization errors using the PEDCHECK software (O'Connell and Weeks, 1998), and no errors were detected.

Joint linkage and association analyses

With recent trends toward large-scale genetic association studies, the need for large sample sizes to detect small effects in psychiatric genetic studies continues to increase. However, the majority of analytic approaches have been limited to either a family-based or a case-control design, resulting in the lack of synthesis of data from multiple studies. In this study, we adopt a unified strategy combining data from both family and case-control samples. DNA samples were available from both parents and offspring for a considerable proportion of families, allowing for testing of genetic linkage to mood disorder. To maximize power, we utilized all samples including singletons to carry out a joint linkage and association analysis using PSEUDOMARKER v 1.0.5 (please visit <http://www.helsinki.fi/~tsjuntun/pseudomarker/>) (Lathrop and Lalouel, 1984; Lathrop *et al.*, 1984; Lathrop *et al.*, 1986; Cottingham *et al.*, 1993; Schaffer *et al.*, 1994; Goring and Terwilliger, 2000b; Hiekkalinna *et al.*, 2011a; Hiekkalinna *et al.*, 2011b).

Pseudomarker is a likelihood-based statistical method that enables analysis of multiple data structures in a unified manner. Both the affected and the unaffected samples are included in the likelihood model. Briefly, Pseudomarker uses the ILINK program that is part of the

LINKAGE package (Terwilliger and Ott, 1994; Ott, 1999), a classical method to test for linkage. In this method, for the null hypothesis, we maximize the likelihood over marker allele frequencies under the assumption of no linkage. To test for linkage, we would compare this with the likelihood maximized over marker allele frequencies and the recombination fraction jointly (i.e. the classical LOD score test, with allele frequencies as a nuisance parameter). To add the linkage disequilibrium (LD) component, we then maximize the likelihood over the recombination fraction jointly with conditional allele frequencies at the marker (conditional on the disease locus allele on the same haplotype) under a parametric penetrance model for disease. The joint test of linkage and LD is a comparison of the likelihood with both linkage and LD versus no linkage and no LD. *P*-values from this test reject the null of ‘no linkage and no association’, but certainly alone cannot tell which of those two phenomena is the cause for rejection of the null hypothesis. Pseudomarker is based on the classic linkage approach used decades ago, with the exception that Pseudomarker carries out the analyses in a computationally reasonable time, having modeled LD as conditional allele frequencies of markers given disease, rather than as haplotype frequencies without constraint.

The Pseudomarker approach approximates a ‘model-free’ affected-relative-pair analysis, but maintains an important property of LOD score analysis – that is, the pedigree correlations between all relatives are considered jointly, and the pedigree is not broken into a set of all possible relative pairs (Goring and Terwilliger, 2000a, 2000b). As the true mode of inheritance for mood disorders is unknown, both dominant and recessive models were used in our analyses. According to the Pseudomarker, recommended model parameterization (Goring and Terwilliger, 2000b), high penetrances with no phenocopies, and low disease allele frequencies were assumed for both the dominant and the recessive genetic models considered. These models were used because they have been shown to provide the greatest power to detect linkage (Terwilliger, 2001). In the absence of any firm knowledge of the mode of inheritance of the disease, as is typically the case for complex diseases, application of a limited set of simple genetic models has been shown to work well when testing for linkage (Greenberg *et al.*, 1998; Abreu *et al.*, 1999).

Although population stratification is not a major concern in family-based studies (Gray-McGuire *et al.*, 2009), it is a commonly cited cause of false-positive findings in case-control association studies (Knowler *et al.*, 1988). This likelihood of increased type I error rate has led to the development of many methods that mitigate the effects of population stratification including ‘genomic control’ (Devlin and Roeder, 1999; Pritchard and Rosenberg, 1999; Gorroochurn *et al.*, 2006), with demonstrable limitations (Gray-McGuire *et al.*, 2009). Although population stratification may influence analyses of the singleton data, we have obviated this by including only samples of Caucasian ancestry as adjustment for population stratification within PSEUDOMARKER was not feasible.

Pathway analysis

The associated candidate genes were examined to elucidate biological pathways that may be involved in mood disorders by the Ingenuity Pathways Analysis (IPA) software (Ingenuity Systems, <http://www.ingenuity.com>) utilizing an unsupervised analysis. To build networks, IPA queries the Ingenuity Pathways Knowledge Base for interactions between the identified (focus) genes in our study, the 28 genes that were found to be associated with mood disorder in the present study, and all other gene objects stored in the knowledge base to generate a set of networks with a maximum network size of 35 genes/proteins. Networks are shown graphically as genes/gene products (nodes) and the biological relationships between the nodes (edges). All edges are supported by at least one reference from the literature or from canonical information stored in the Ingenuity Pathways Knowledge Base. In addition, IPA

generates a score for each network according to the fit of the user's set of significant genes. The score represents the relative ranking of the network on the basis of the following criteria, optimizing for both gene–gene interconnectivity and the number of user-supplied 'focus' genes while maintaining the network size constraint. The genes used for pathway analysis (shown in Table 1) were selected on the basis of the significance of linkage-association findings with a type I error rate of α 0.05 or less chosen *a priori*.

Gene expression profiling

As part of our ongoing large-scale gene expression study, we have gene expression data from the DLPFC and AC, independent of 21 cases with mood disorders and 25 nonpsychiatric controls. These brain regions were examined because they have been implicated previously in the neuropathology of mood disorders (Milak *et al.*, 2005). Post-mortem tissue from Brodmann areas 9 (BA9) and 24 (BA24) were dissected from frozen brain sections that had been transferred from -80 to -20°C for 2 h. BA were identified using gyral and sulcal landmarks, cytoarchitecture, and a standardized coronal atlas as described previously (Arango *et al.*, 1995). Blocks were sectioned with a cryostat at $200\ \mu\text{m}$ (-20°C). Meninges and white matter were removed as much as possible during sectioning, before collection into microtubes. Two hundred microgram-thick sections were collected in microtubes at -20°C . Total RNA was extracted using the TRIZOL method (Invitrogen, Carlsbad, California, USA), with RNA quality assessed by analysis on an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, California, USA). For all samples, the RNA was of high quality, with average RIN of at least 7.265 (minimum = 6.5; maximum = 9.1). Only samples with RIN more than 6.0 were used in further gene expression assays. RNA samples were primed with a standard T7-oligo(dT) primer and cDNA synthesis was carried out using $5\ \mu\text{g}$ of total RNA according to the Affymetrix manufacturer's protocol (<http://www.affymetrix.com/support/>). Amplified antisense RNA (aRNA) was produced using in-vitro transcription directed by T7 polymerase. Fifteen micrograms of the purified and fragmented aRNA were hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 (HGU133Plus2.0) arrays. All microarrays had a high quality on the basis of 5' : 3' GAPDH integrity ratios calculated using GCOS software (downloaded from Affymetrix). All samples were processed at the same time, containing both cases and matched controls. Probeset-level signal intensities were extracted using the Robust Multiarray Average algorithm (Galfalvy *et al.*, 2003; Irizarry *et al.*, 2003) from the R affy package from Bioconductor project website (<http://www.bioconductor.org>). Brain pH and post-mortem interval showed no correlation with disease status or gene expression ($P = 0.75$ and 0.98 , respectively). All the probesets from the HGU133Plus2.0 array corresponding to the genes with significant linkage-association findings, as well as those identified through pathway analysis, were selected, and the fold change values were recorded for each of the probesets. Fold change is defined as the ratio of the average expression level in the experimental (mood disorder) group to the average expression level in the control group; equal group means would correspond to a fold change of 1. As each gene is represented by several probesets, we chose to report only the most extreme fold change per gene (i.e. the one farthest from a unit fold change in either direction). Genes and corresponding probes with detectable expression intensities across more than 90% of the samples were included in this analysis. We also carried out a two-sample *t*-test to detect significant expression differences in mood disorder case versus control groups for each brain region separately. For each gene, the normalized probe-set expression intensities were used to test for gene expression differences. Significance levels are reported as a descriptive statistic and were used to filter the gene expression results in determining the relative support of the gene expression data for the primary linkage-association findings.

Results

As part of this large-scale candidate gene analysis for mood disorder, we identified suggestive evidence for linkage association for 28 of the total 130 genes (with per-locus *P*-values = 0.0002–0.048; Table 1). Applying the Benjamini–Hochberg linear step-up procedure (Benjamini and Hochberg, 1995) to correct for multiple testing, we found that none of these loci remained significant at the 0.05 significance level. However, many of these findings are consistent with previous linkage and association reports, and together with genetic pathway and functional analyses, they show a number of novel findings. Consistent with previous reports, we identified the following genes associated with mood and depressive disorders (Table 1), including protein kinase C, ϵ (*PRKCE*) (Costas *et al.*, 2010), brain-derived neurotrophic factor (*BDNF*) (Licinio *et al.*, 2009; Lavebratt *et al.*, 2010; Mata *et al.*, 2010), *NTRK2* (Dong *et al.*, 2009), and *GRM1* (Tsunoka *et al.*, 2009). We also identified novel genes with significant linkage association, which have not been reported previously to have a significant linkage or association with mood disorders. These include arginine vasopressin receptor 1A (*AVPR1A*), *OPRD1*, *SLC6A11*, *GABA A* receptor, $\gamma 2$ (*GABRG2*), glutamate receptor, ionotropic, kainate 1 (*GRIK1*), catalase (*CAT*), neurotensin receptor 1 (high affinity) (*NTSR1*), alcohol dehydrogenase 7 (class IV) (*ADH7*), and vesicular inhibitory amino acid transporter (*VIAAT*) (Table 1).

We further examined these genes using both pathway and gene expression analyses. We carried out pathway analysis to identify gene–gene interactions using the IPA to highlight relationships among genes and identify common cellular pathways (Fig. 1). We also examined brain gene expression patterns across the dorsolateral prefrontal cortex and AC for those 28 genes with significant linkage association in our data (Table 2), and combined these data with the pathways identified by IPA to inform our understanding as to how these genes may be involved in the neurocircuitry of mood disorders. Specifically, the IPA showed interactions between the protein products of the associated candidate genes (Fig. 1) and the gene expression analysis (Table 2), indicating the potential functional role of these genes in the pathogenesis of mood disorders. In this way, we identified candidate genes involved in a number of critical pathways, which can be important in brain function in a complex network of gene–gene interactions. Specifically, we found two genes that had evidence in all three domains, genotype, expression, and pathway analysis: the *NTRK2* (*TRKB*) gene, a receptor of *BDNF* that showed linkage association and lower expression, and the opioid receptor, $\kappa 1$ (*OPRK1*), where there was both increased expression in the DLPFC of patients with mood disorder and evidence of linkage association to mood disorder.

Discussion

This study aimed to identify convergent lines of evidence for candidate genes for mood disorders using linkage-association analysis, gene expression profiling in post-mortem cortical samples, and pathway analysis. Several candidate genes were supported by all three lines of investigation. *NTRK2* (*TRKB*) (significant in linkage-association analysis and whose expression was significantly decreased in AC of mood disorder samples) is a receptor for *BDNF*, a node of high connectivity in our network analysis. Both are broadly expressed in the developing and adult mammalian brain. *BDNF/NTRK2*-stimulated intracellular signaling is critical for neuronal survival, morphogenesis, and plasticity (Duman and Monteggia, 2006). Binding of *BDNF* to *NTRK2* induces a number of intracellular signaling pathways, including mitogen-activated protein kinase/extracellular signal-regulated protein kinase (*MAPK/ERK*), phospholipase C γ (*PLCg*), and phosphoinositide 3-kinase (*PI3K*) pathways. *BDNF* appears to be low in major depression (Dell'osso *et al.*, 2010; Shi *et al.*, 2010) and suicide in youth (Pandey *et al.*, 2010). The genes *BDNF* (Licinio *et al.*, 2009; Lavebratt *et al.*, 2010; Mata *et al.*, 2010) and *NTRK2* (Dong *et al.*, 2009; Schosser *et al.*, 2011) have been

associated previously with major depressive disorder, although no association was detected in a depression meta-analysis study of a large cohort examining the *BDNF* val66met polymorphism (Chen *et al.*, 2008).

All three lines of investigation support a role for the opioid receptors, specifically *OPRK1*, in the pathogenesis of mood disorders. The *OPRK1* transcript showed both increased expression in the DLPFC of patients with mood disorder and evidence of linkage-association in our mood disorder cohort. The network analysis showed that *OPRK1* interacts with multiple downstream effectors of *BDNF*. It has been reported previously that κ opioid receptor agonists exert an antidepressant-like effect (Zhang *et al.*, 2007; Carr *et al.*, 2010; Knoll and Carlezon, 2010). In addition, κ opioid receptor agonists upregulate *BDNF* expression, supporting this interaction (Akbarian *et al.*, 2002; Torregrossa *et al.*, 2006; Zhang *et al.*, 2006; Zhang *et al.*, 2007). No previous genetic associations have been reported between *OPRK1* and mood disorders, and as such, these data represent a novel finding.

Furthermore, proenkephalin (*PENK*), which produces endorphins that stimulate the opioid receptors, also showed evidence of linkage association to mood disorders and lower expression in the post-mortem DLPFC samples from patients with mood disorder in this study (Table 2). The *PENK* gene has been found to be associated with bipolar disorder (Ogden *et al.*, 2004). In addition, inhibition of enkephalinase, which degrades enkephalins, produces an antidepressant effect in mice, which is mediated by the opioid receptors, providing further evidence for a role of the opioid system in mood disorder (Jutkiewicz *et al.*, 2006). The increased *OPRK1* expression in the DLPFC of our patients with mood disorder may represent a compensatory mechanism for the decreased *PENK* expression. The suggestive genetic linkage association of both *OPRK1* and *OPRD1*, and *PENK* with mood disorders in our separate mood disorder cohort (Table 1) supports a role for dysfunction of the opioid system in mood disorder and suggests that this is not an effect of pharmacological or other environmental factors on gene expression. Dysregulation of this subset of opioid receptor signaling may provide clues to the comorbidity and potential interrelated pathogenesis of these disorders.

The primary strength of this study is the use of convergent lines of evidence from genetic linkage-association studies, network analysis showing connectivity of implicated candidates, as well as gene expression profiling of candidates in post-mortem samples. However, these findings should be viewed with caution, warranting replication in future studies. Although we observed significant per-locus linkage-association results for a number of candidate genes, these results were not significant after correction for multiple testing. As most candidate genes for mood disorders have a small effect, large enough sample sizes required to detect these effects are often unobtainable, a problem common to most studies in this field. To compensate for this shortcoming, we focused our efforts on those candidate genes that produced suggestive linkage-association signals and then proceeded to interpret the biological significance of these findings using pathway and gene expression analyses. This is a compelling strategy in that it does not strictly rely on the extent of statistical signal obtained from genetic linkage-association analyses, which, in neuropsychiatric disorders, are typically weak and difficult to replicate. Rather, this strategy also relies on knowledge from genetic pathway interactions that underscore the importance of specific pathways with multiple possible points of dysregulation in mood disorders. It also uses an independent measure of gene function that can reflect both genetic and epigenetic effects, as well as the impact of transcription factors whose genes are also being evaluated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

A graphical representation of the interactions between the products of significant candidate genes in the top network to which most of our candidate genes belonged in the ingenuity database. Molecules are placed in their cellular compartments, which are listed on the left (e.g. nucleus, cytoplasm), and those that do not have a defined compartment are placed outside of the black box. The candidate genes that were significant in our analysis are in grey and the key on the right indicates the functional categories of the proteins identified in the network. ADCY, adenylate cyclase; *ADPR1A*, adrenergic α -1A receptor; *ADRA1A*, adrenergic α -1A receptor; *ADRA2B*, adrenergic α -2B receptor; *BDNF*, brain-derived neurotrophic factor; *CHRM3*, cholinergic receptor, muscarinic 3; *CHRNA4*, cholinergic receptor, nicotinic, α 4; *DDC*, dopa decarboxylase (aromatic L-amino acid decarboxylase); *DRD2*, dopamine receptor D2; ERK, extracellular signal-regulated protein kinase; GPCR, G protein-coupled receptors; *GRM1*, glutamate receptor, metabotropic 1; *HTR2B*, 5-hydroxytryptamine (serotonin) receptor 2B; IL-1, Interleukin-1; *MAOB*, monoamine oxidase B; *MAPK*, mitogen-activated protein kinase; *NF κ B*, nuclear factor κ B; *NGF* β , nerve growth factor β ; *NMDA*, N-methyl D-aspartate; *NTRK2*, neurotrophic tyrosine kinase receptor, type 2; *OPRD1*, opioid receptor, δ 1; *OPRK1*, opioid receptor, κ 1; *OXT*, oxytocin; *PENK*, proenkephalin; Pkc(s), protein kinase Cs; *PI3K*, phosphoinositide 3-kinase; *PLC* γ , phospholipase C γ .

Table 1
Genes significantly associated with mood disorder under either dominant (D) or recessive (R) genetic models

Gene ID	Gene name	Chromosome	SNP	Model	P-value
<i>CHRM3</i>	Cholinergic receptor, muscarinic 3	1	rs621060	D	0.0002
<i>DDC^a</i>	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	7	rs4947510	R	0.004
<i>NGFB^{b,c}</i>	Nerve growth factor (β polypeptide)	1	rs2239622	R	0.004
<i>PENK</i>	Proenkephalin	8	rs11998459	R	0.006
<i>OPRD1^d</i>	Opioid receptor, $\delta 1$	1	rs529520	R	0.006
<i>AVPR1A^e</i>	Arginine vasopressin receptor 1A	12	rs3803107	R	0.008
<i>CAT</i>	Catalase	11	rs564250	D	0.013
<i>ALDH2</i>	Aldehyde dehydrogenase 2 family (mitochondrial)	12	rs7311852	R	0.017
<i>PRKCE</i>	Protein kinase C, ϵ	2	rs585156	D	0.018
<i>OPRK1</i>	Opioid receptor, $\kappa 1$	8	rs7817710	D	0.019
<i>BDNF</i>	Brain-derived neurotrophic factor	11	rs12273363	D	0.021
<i>SLC6A11</i>	Solute carrier family 6 (neurotransmitter transporter, GABA), member 11	3	rs2272399	D	0.022
<i>GRIN2B</i>	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	12	rs2193511	D	0.025
<i>NTRK2</i>	Neurotrophic tyrosine kinase receptor, type 2	9	rs893584	D	0.027
<i>NTSR1</i>	Neurotensin receptor 1 (high affinity)	20	rs3787535	D	0.028
<i>ALDH1A1</i>	Aldehyde dehydrogenase 1 family, member A1	9	rs11143429	R	0.030
<i>ADH7</i>	Alcohol dehydrogenase 7 (class IV)	4	rs729147	D	0.033
<i>VIAAT</i>	Vesicular inhibitory amino acid transporter	20	rs1322183	D	0.033
<i>OXT</i>	Oxytocin, prepropeptide	20	rs2740210	R	0.034
<i>GRM1</i>	Glutamate receptor, metabotropic 1	6	rs7770466	R	0.036
<i>HTR2B^f</i>	5-hydroxytryptamine (serotonin) receptor 2B	2	rs3806545	R	0.036
<i>DRD2</i>	Dopamine receptor D2	11	rs2075652	D	0.036
<i>GRIK1</i>	Glutamate receptor, ionotropic, kainate 1	21	rs467407	D	0.037
<i>ADRA2B</i>	Adrenergic, α -2B receptor	2	rs2229169	R	0.037
<i>ADRA1A</i>	Adrenergic, α -1A receptor	8	rs10503800	D	0.038
<i>GABRG2</i>	γ -aminobutyric acid A receptor, $\gamma 2$	5	rs3219203	D	0.040
<i>CHRNA4</i>	Cholinergic receptor, nicotinic, $\alpha 4$	20	rs755203	D	0.044
<i>MAOB</i>	Monoamine oxidase B	X	rs5905512	R	0.048

Additional single nucleotide polymorphisms (SNPs) that were statistically significant:

- ^a Gene: *DDC*, SNP: rs3779084, model: D, *P*-value = 0.045.
- ^b Gene: *NGFB*, SNP: rs6537860, model: R, *P*-value = 0.004.
- ^c Gene: *NGFB*, SNP: rs2268793, model: D, *P*-value = 0.020.
- ^d Gene: *OPRD1*, SNP: rs204055, model: R, *P*-value = 0.008.
- ^e Gene: *AVPR1A*, SNP: rs3021528, model: R, *P*-value = 0.036.
- ^f Gene: *HTR2B*, SNP: rs17586428, model: D, *P*-value = 0.038.

Genes differentially expressed in the dorsolateral prefrontal cortex and anterior cingulate of post-mortem cases with mood disorder and nonpsychiatric controls

Table 2

Gene ID	Gene name	Brain region	Fold change case vs. control	P-value	Case mean (SD)	Control mean (SD)
<i>CAT</i>	Catalase	AC	1.085	0.001	3.961 (0.1311)	3.843 (0.100)
<i>OPRK1</i>	Opioid receptor, κ1	DLPFC	1.209	0.012	6.905 (0.3777)	6.632 (0.370)
<i>NTRK2</i>	Neurotrophic tyrosine kinase receptor, type 2	AC	0.818	0.024	7.457 (0.500)	7.747 (0.382)
<i>MAOB</i>	Monoamine oxidase B	DLPFC	1.085	0.042	10.452 (0.198)	10.307 (0.205)
<i>PENK</i>	Proenkephalin	DLPFC	0.729	0.042	7.432 (0.756)	7.889 (0.802)
<i>MAOB</i>	Monoamine oxidase B	AC	1.085	0.050	3.175 (0.159)	3.098 (0.111)

Average normalized expression values along with SD for mood disorder (case) and control samples are reported.
AC, anterior cingulate; DLPFC, dorsolateral prefrontal cortex.