

Figure 2. Haplotype block structure of *HCRTR1* in the Japanese population. The numerical values in each cell represent a linkage disequilibrium parameter D' ($\times 100$), whereas no value presents $D' = 1$. The cell color is graduated according to the strength of linkage disequilibrium between markers, which is defined by both the D' value and the confidence bounds on D' (Barrett et al 2005).

typic distributions for all examined polymorphisms conformed to the Hardy-Weinberg equilibrium in all the sample groups, except for the Ile408Val of *HCRTR1* in the polydipsia group ($p = .019$). Because the integrity of the genotypes was confirmed by sequencing of both DNA strands, this deviation suggests that the genetic variation or a nearby polymorphism(s) in tight linkage disequilibrium with the Ile408Val might be causally associated with polydipsia.

To define the genomic boundaries of the potential risk region encompassing the 1408V variant of *HCRTR1*, we constructed haplotype blocks by using Japanese data from the HapMap project (Figure 2). A genomic stretch of approximately 40 kilobases (kb) encompassing the *HCRTR1* gene (approximately 8 kb) represents one haplotype block, with single nucleotide polymorphisms rs3753612 (-2384C>G), rs968335 (IVS2+465G>A), and rs2271933 (1408V) in complete linkage disequilibrium with each other (Figure 2).

Ca²⁺ Influx Through HCRTR1

Both the wild-type and polymorphic receptor mRNAs showed an almost identical secondary structure with the same minimum free energy of -495.41 kcal/mol; however, the location of the HCRTR1 408Val mutation in the cytoplasmic tail and therefore potential binding site for G proteins, and the increased phosphorylation potential of the immediately adjacent serine residue when the variant position is valine (.899 vs. .949), could alter signal transduction in carrier cells on activation of the receptor (Figure 3A). This prompted us to examine the potential of the mutant receptor to modulate intracellular calcium in response to HCRT1, using COS7 cells transiently expressing HCRTR1. Figure 3B shows that both the normal and mutant receptors elicit a dose-dependent response to HCRT1 in the range tested. There was no significant difference between the receptors in terms of the speed or magnitude of response. In immunofluorescence experiments, both the normal and mutant receptors displayed a

similar pattern of localization to the cell surface of COS7 cells and the SK-N-SH neuroblastoma cell line (data not shown).

Discussion

Despite the significant morbidity of polydipsia, little is known about its pathophysiology, which is probably multifactorial. A small familial study suggests that the disorder could have a genetic basis, whereas other studies put forward dopamine dysfunction as a contributory factor (Shinkai et al 2003; Verghese et al 1993). Our data showing that the *ACE I/D* polymorphism, a regulator of AT1I levels, does not play a role in the development of polydipsia is in keeping with the findings of Shinkai et al (2003). They do, however, find that the D allele shows a trend of association with a more severe stage of polydipsia, water intoxication. Both studies contrast with a report from Ouyang et al (2001), who found a trend of association of the I allele with polydipsia. This study, however, might have been hampered by a small sample size (18 polydipsic subjects). None of the studies showed association of the *ACE I/D* polymorphism with overall schizophrenia.

In this analysis of the orexin signaling system, our data suggest that the 408Val mutation in *HCRTR1* might predispose carriers to polydipsia, although the variant is not associated with

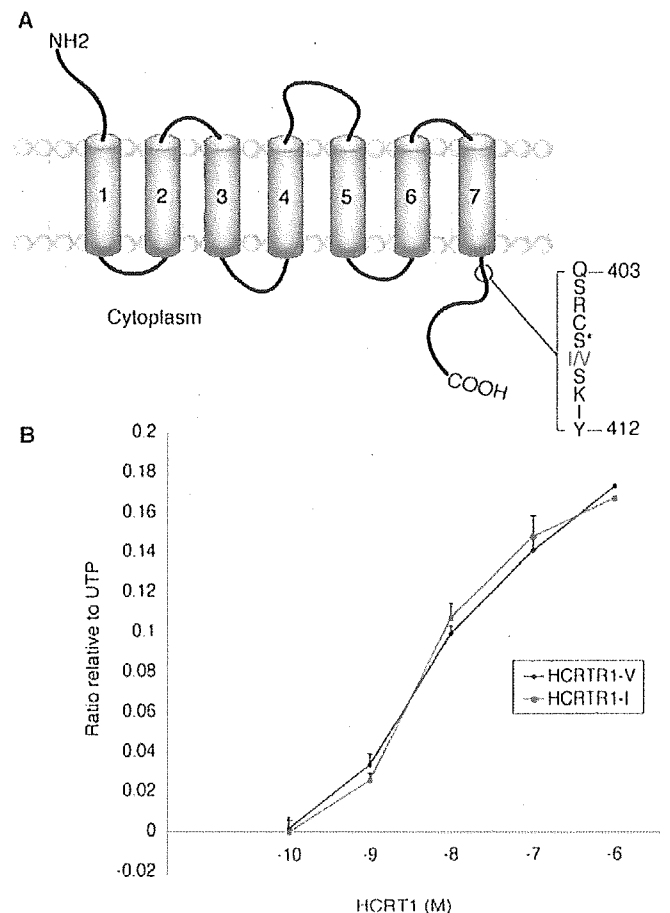


Figure 3. (A) Diagram showing the structure of HCRTR1. The position of the Ile408Val mutation is shown in red. The asterisk (*) denotes the potentially phosphorylated serine residue. (B) HCRT1 concentration-dependent increase in intracellular calcium release in COS7 cells transiently expressing normal and mutant HCRTR1. Results are expressed as a ratio against UTP calcium mobilization and are given as mean \pm SEM, where $n = 5$.

the general development of schizophrenia. This suggests that although the disorders coexist, and both show altered dopamine regulation, there remain unique contributory factors. The greatest contrast in mutation frequency is between polydipsic schizophrenic and nonpolydipsic schizophrenic subjects. A plausible explanation for this is that the control group contains subjects who, with or without additional genetic hits, could potentially go on to develop polydipsia upon exposure to neuroleptic drug treatment, whereas the nonpolydipsic schizophrenic group only contains subjects who are refractive to developing polydipsia, even after neuroleptic treatment. Although there were twice as many male as female polydipsic subjects, the frequency of the 408Val allele was comparable between the genders for all sample groups (frequency differences between the genders $\leq 1\%$). Thus, the different gender ratio is unlikely to have a bearing on these results. The increased prevalence of polydipsia in male schizophrenic subjects is a previously noted but uninvestigated phenomenon (Ahmed et al 2001; Kawai et al 2001). Intriguingly, the orexin ligand and receptors show sexually dimorphic expression in rats, with higher HCRT and HCRTR1 expression in the female hypothalamus compared with males and higher levels of HCRTR2 in male adrenal glands compared with females (Johren et al 2001, 2002).

Conventional neuroleptics with a high affinity for dopamine D2 receptors are known to induce polydipsia in schizophrenic subjects, whereas atypical drugs, such as clozapine, that spare D2 receptors and additionally target serotonergic receptors relieve the symptoms of polydipsia. Interaction between the orexin and dopaminergic systems in terms of physical juxtapositioning and co-expression of the receptor is well documented. Nakamura et al (2000) showed that Orexin-induced hyperlocomotion and stereotypy are mediated through the dopaminergic system, more specifically the D1 and D2 receptors (see also Uramura et al 2001). In addition, orexin A can activate serotonergic neurons, and serotonin and its agonists hyperpolarize orexin neurons through the 5-HT1A receptor (Muraki et al 2004). In a study of antipsychotic drug-related weight gain, clozapine, which induces weight gain, was found to differentially activate not only dopaminergic but also subsets of orexin neurons in the lateral hypothalamus, prefrontal cortex, and perifornical areas relative to drugs that do not induce significant weight gain (Fadel et al 2002). Therefore, orexin neurons are probably implicated in at least one adverse effect of neuroleptic treatment, and polydipsia might represent a second condition mediated by these neurons. After repeated exposure to neuroleptics, abnormal orexin signaling might increase dopaminergic tone in selected neural circuits, disturbing secondary targets and leading over a period of time to compulsive fluid ingestion.

The 408Val mutation was first reported as a "benign polymorphism" in human narcolepsy (Peyron et al 2000) and has been identified in a neuroblastoma cell line, SK-N-SH (Wieland et al 2002). Measurements of intracellular calcium failed to detect a difference between the normal and mutant receptors after treatment with orexin A. It is possible that an overexpression assay lacks the required sensitivity in this instance. Measurements from single cells that naturally carry the mutation, such as SK-N-SH, could prove more informative. Because valine is the corresponding amino acid in a variety of species, such as dog, mouse, rat, and chicken, it is also possible that the 408Val mutation is nonfunctional but lies in linkage disequilibrium with an undetected functional variant. The *HCRTR1* gene resides in a haplotype block of approximately 40 kb that overlaps with a neighboring gene, *PEFLIN* (*PEF*). This gene lies less than 3 kb

downstream of *HCRTR1* and therefore would warrant further genetic analysis for functional variants in future studies.

How orexin signaling might contribute to the development of polydipsia is difficult to understand at this stage, because it is clear that there are heterogeneous populations of orexin neurons that interact with the dopaminergic system through various positive and negative feedback loops and that non-orexin neurons also express orexin receptors (Backberg et al 2002; Fadel et al 2002). Although the *HCRTR1* 408Val mutation shows strong association with polydipsia, these results need to be replicated in independent samples. If confirmed, schizophrenic mutation carriers could be selected for alternative treatments, such as clozapine, which has a greater toxicity but proven efficacy against excessive water consumption and schizophrenia (Spears et al 1996). For current polydipsic subjects, new adjunct therapies could be designed with HCRTR1 antagonists, such as SB-334867 (Rodgers et al 2001). It is important to conduct further studies to examine whether the orexin system could represent a new target for the development of drugs against polydipsia.

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Supplementary material cited in this article is available online.

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REVIEW ARTICLE

Genetic tests of biologic systems in affective disorders

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To liberate candidate gene analyses from criticisms of inexhaustiveness of examination of specific candidate genes, or incompleteness in the choice of candidate genes to study for specific neurobiological pathways, study of sizeable sets of genes pertinent to each putative pathophysiological pathway is required. For many years, genes have been tested in a 'one by one' manner for association with major affective disorders, primarily bipolar illness. However, it is conceivable that not individual genes but abnormalities in several genes within a system or in several neuronal, neural, or hormonal systems are implicated in the functional hypotheses for etiology of affective disorders. Compilation of candidate genes for entire pathways is a challenge, but can reasonably be carried out for the major affective disorders as discussed here. We present here five groupings of genes implicated by neuropharmacological and other evidence, which suggest 252 candidate genes worth examining. Inexhaustiveness of gene interrogation would apply to many studies in which only one polymorphism per gene is analyzed. In contrast to whole-genome association studies, a study of a limited number of candidate genes can readily exploit information on genomic sequence variations obtained from databases and/or resequencing, and has an advantage of not having the complication of an extremely stringent statistical criterion for association.

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Identifying susceptibility genes has long been challenging in studies of major affective disorders, as well as in other common complex diseases such as schizophrenia, asthma, diabetes, and cardiovascular diseases. Association study, which typically examines differences in allele frequency of a genetic marker between cases (affecteds) and controls, remains a major approach of disease gene mapping, and has been employed to examine possible roles of candidate genes in the etiology of a disease of interest.

Genomewide linkage analysis, in contrast, does not limit itself to a particular genomic region, apparently avoiding the risk of overlooking any genes with poor or even no information on biological functions. For more than two decades, linkage mapping has proven to be remarkably effective to guide researchers to numerous disease genes, each predisposing to a Mendelian trait. Also, development of computer algorithms for model-free linkage analysis has considerably facilitated appropriate genetic dissections of complex phenotypes with unknown mode of inheritance. In studies of major psychiatric illnesses,

evidence of linkage has led to the detection of associations of specific genes with illness: dysbindin 1 (DTNBP1)¹ on chromosome 6p and neuregulin 1 (NRG1)² on 8p for schizophrenia, and G72/G30 on 13q for both schizophrenia³ and bipolar disorder.⁴ These genes have been demonstrated to be associated with schizophrenia and/or bipolar disorder in multiple independent data sets.^{5–8} However, model-free linkage analysis is unlikely to detect genes with very weak effects (modest increase in probability of illness, given the associated allele). This has led to a resurgence of association analysis because of its much higher statistical power, particularly in the studies of complex diseases, where multiple genes are considered to exert weak effect along with environmental factors.⁹

Candidate gene association studies have historically been plagued by nonreplication. A recent meta-analysis of genes that had a large number of association studies emphasized possible contribution of false-negative underpowered studies to inconsistent results, and suggested consistent weak effects of the genes for serotonin receptor 2A (HTR2A) and dopamine receptor D3 (DRD3) on susceptibility to schizophrenia.¹⁰ Thus, inconsistencies among reports may be consequences of what previous studies have failed to address. Until recently, it has only been feasible to interrogate a few genes in particular systems, and these interrogations have often been

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limited to very few polymorphisms in a limited number of subjects, because of limitations in the costs of genotyping, and in the knowledge of the human genome. Advances in genomics and bioinformatics, in high-throughput genotyping, in statistical analysis, and in the availability of large samples of patients with well-defined phenotypes, as well as comparable numbers of matched controls, can be expected to enhance the likelihood of detection of valid associations.

What remains as the most serious concern about the paradigm of candidate gene association study is its 'incompleteness' resulting from *ad hoc* selection of candidate genes. *A priori* hypotheses have to be made on the primary cause of the disease being studied, when starting a candidate gene study. For many years, genes have usually been tested in a 'one by one' manner for association with major affective disorders, primarily bipolar illness. However, it is also conceivable that not individual genes but abnormalities in several genes within a system or in several neuronal, neural, or hormonal systems are implicated in the functional hypotheses for etiology of affective disorders. Analysis of entire systems examining a same sample set has only rarely been undertaken,^{11,12} and examples of definitive success have yet to be seen. At this time, analysis of a well-chosen and comprehensive set of candidate genes, with the support of informatics analysis of the genomic structure of each gene, may yield successful detection of specific genes and pathways associated with illness.

In the following sections, we first discuss the advantage of a hypothesis-based systematic association study on a limited number of candidate genes in contrast to the whole-genome association study, and subsequently demonstrate that, in the case of affective disorders, compilation of candidate genes pertinent to each major neurobiological system suggested for susceptibility can be reasonably carried out. The idea of testing systems can be generalized to studies of other common complex diseases.

Systematic candidate gene study vs whole-genome association study

Association studies are intended to capture linkage disequilibrium (LD) between genotyped markers and a disease causal variant, including when the marker being genotyped happens to be exactly the causal variant. Recent studies have revealed that chromosomal segments, spanning a few to hundreds of kilobases, can often be represented by only a few haplotypes because of strong underlying LD that associates specific alleles at each polymorphic site in the segment. Such a segment is called a 'haplotype block' and the analysis of a block can be achieved by typing a small set of SNPs (haplotype tag SNPs or htSNPs) that are most informative for discriminating haplotypes.^{13,14} The International HapMap project¹⁵ (see Electronic-Database Information) has just com-

pleted genotyping of a million SNPs in four different populations with the aim of providing information on genomic variations including the extent of LD, haplotype blocks and htSNPs. This modeling of genomic variations especially favors the idea of whole-genome LD mapping, which aims to locate disease susceptibility variants using a set of limited number of markers across the entire genome.

However, it is open to question how informative the limited number of SNPs can be in terms of sequence variations of the genome. First, it is unclear to what degree the entire genome can be captured by block-like structures. Since haplotype blocks reflect underlying LD, whose extent varies considerably across the genome, short blocks may become evident only by highly dense genotyping. According to a simulation under a recombination hot-spot model by Wall and Pritchard,¹⁶ even genotyping with a marker density obtained by resequencing would capture only up to 71% of the entire genome as blocks. Besides, gene conversion, which is not incorporated into this model, seems to have generated discrepancies between haplotype block fractions observed in actual data and predicted by simulations. Gene conversion can give rise to a 'hole' in an LD or a haplotype block, and a susceptibility variant in such a hole is likely to be overlooked. Secondly, even when a haplotype block is evident, it is unclear if markers from the databases capture sufficient haplotype diversity. For example, a haplotype with an estimated frequency of 45% may really be a group of three haplotypes each with a frequency of 15%. This loss of information can substantially reduce the power of detecting association depending on the frequency of a causal variant. Selecting the most informative markers not depending on the haplotype block model, as suggested in Carlson *et al*,¹⁷ would considerably circumvent these problems. Resequencing of genomic regions of interest will also be necessary (see Electronic-Database Information for current examples). From the viewpoint of the number of SNPs to be genotyped, these approaches look feasible for a study of a limited number of candidate genes, but not for whole-genome LD mapping or for its gene-focused form.^{9,18,19}

Since the prior probability of association for a biologic candidate gene can be expected to be considerably higher than that for a gene randomly picked up from the genome, candidate gene approach may benefit from increased statistical power by analysis controlling 'false discovery rate'.²⁰ Even in the conventional statistical tests (eg Bonferroni procedure) for multiple hypotheses, which control overall type I error rate, the study of limited number of candidates derived from a few hypotheses would not suffer from the complication of a very stringent statistical criterion for association, because the number of markers would be less than in a whole-genome LD mapping. Also, a method has recently been developed to detect a set of associated genes, which may statistically interact with each other.²¹ Interpretation of results of this analysis can be more

straightforward when we study multiple genes with known biologic functions. Thus, whole-genome association study does not replace the candidate gene approach using a sufficient number of informative markers, particularly when we are anxious about missing associations.

Genetic testing of functional systems (pathways) in major affective disorders

Since the first monoamine hypothesis (Figure 1) of depression, based on biochemical pharmacology of antidepressants and reserpine, numerous hypotheses of dysregulation of functional systems in mood disorders have been suggested, but so far no consensus has been reached on any primary molecular mechanism underlying mood disorder susceptibility. Nonetheless, the choice of several systems (Table 1) over others for intensive genetic study can be supported by their relevance to clinical features as

Figure 1 Evolution of 'monoamine hypothesis'. A major hypothesis for the biology of depression was developed in the 1960s, initially proposing that depletion of norepinephrine (NE), and later proposing depletion of serotonin (5-HT) and dopamine (DA), underlie the illness.¹⁰⁰ This 'monoamine hypothesis' was proposed because of the clinical observation that depression often occurs in subjects taking reserpine, an antihypertensive agent, which depletes monoamines from the synaptic vesicles. Also, consistent with the hypothesis was that tricyclic antidepressants and MAO inhibitors were found to increase synaptic monoamine concentrations. The hypothesis was later modified to include alterations of monoamine receptor properties so that it would encompass an explanation for the time (usually days to weeks) required for an antidepressant to take clinical effect despite its immediate action to elevate synaptic monoamine levels.^{101,102} However, either the original or modified form of hypothesis has not been definitively demonstrated so far. Involvement of postsynaptic signaling is now of interest to researchers. A diagram for NE neurotransmission is shown as an example. The postsynaptic receptors for NE are coupled to guanine nucleotide binding proteins (G proteins), which transduce neurotransmitter stimulation to second messenger signaling systems such as cAMP and phosphoinositide pathways. Now that numerous components in the NE neurotransmission system have been identified, including metabolic enzymes, receptors, transporters, and postsynaptic signaling (eg one or more subtypes of G proteins, protein kinase A, protein kinase C, calcium/calmodulin-dependent protein kinases), emphasis is being placed on the broader view of dysregulation in the entire system,¹⁰³ taking interactions of each component into account. Components are presented at the gene level in Table 2. NE: norepinephrine; VMAT: vesicular monoamine transporters; MAO: monoamine oxidases; COMT: catechol-O-methyltransferase; α -AR: α -adrenergic receptors; β 1-AR: beta-1-adrenergic receptor; G: G proteins; AC: adenylate cyclases; PL: phospholipases; PKA: cAMP-dependent protein kinases; PKC: calcium-dependent protein kinases; CREB: cAMP-responsive element binding protein; NET: norepinephrine transporter; DAT: dopamine transporter.

well as by accumulated neurobiological and neuropharmacological findings.

Phenotypic subclasses of the entire spectrum of affective disorders may have different associations with the systems in Table 1. However, we need not assume too much about a specific relationship between systems and subclasses. When samples from different types of affective disorders with abundant clinical records are available, it may be more reasonable to conduct genetic analyses on numerous phenotypic variables after completion of genotyping. Such an approach has successfully been employed in detecting association between the *PDE4D* gene and ischemic type stroke in the analysis of all the samples from broadly defined common forms of stroke.²²

The first step of compiling candidate genes in a given functional hypothesis is to list genes involved in pathways which represent that hypothesis. GO and KEGG databases, for example (see Electronic-Database Information), help overview a set of genes involved in a particular intracellular pathway. To obtain information on genetic components specifically relevant to the phenotypes of interest, intensive literature survey or review is required. There have been a huge number of reports on specific proteins (sometimes specific subtypes) altered in post-mortem brains from bipolar disorder subjects or in brains from rodents treated with mood stabilizers. Also, animal models and systematic expression analyses by microarray or differential display assay provide information on molecules relevant to mood disorders not only at the protein level but also at the gene expression level.

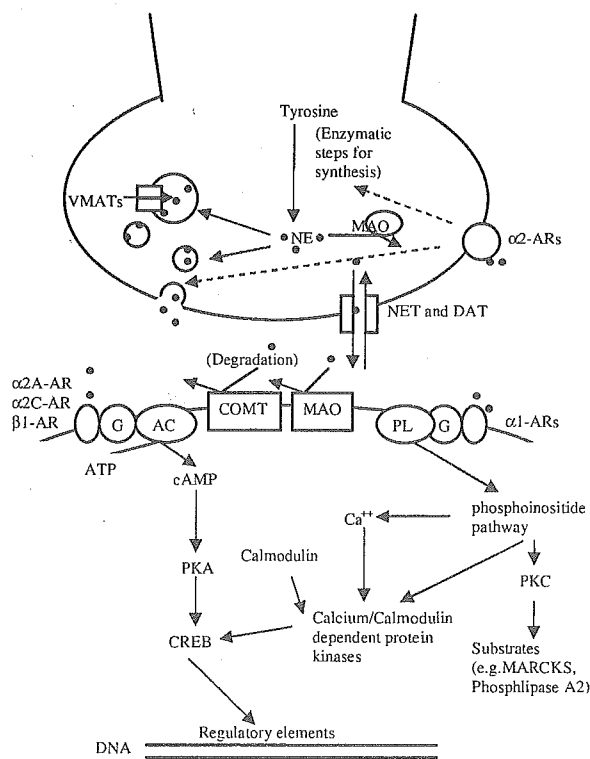


Table 1 Major neurobiological/neuropharmacological systems suggested for roles in major affective disorder

<i>Systems</i>	<i>Subsystems or a group of genes to be analyzed</i>
1. Neurotransmission systems	Monoaminergic neurotransmission (adrenergic, serotonergic and dopaminergic) Cholinergic neurotransmission Amino-acid neurotransmission (GABAergic and glutamatergic) Other neurotransmitter or neuromodulator systems (peptidergic, opioid and others)
2. A neuroendocrine system	HPA axis
3. Neurotrophic and growth factor systems	Neurotrophic/growth factors and shared signaling pathways
4. Circadian rhythm	Clock genes (eg CLOCK, ARNTL1, ARNTL2, CRY1, CRY2) Pathways for entrainment to light/darkness cycle and outputs of suprachiasmatic nucleus (eg ADCYAP1, TGFA, PROK2)
5. Genes implicated in pathophysiology of other diseases relevant to major affective disorders	Parkinson's disease genes (eg PARK2, SCNA, UCHL1) Schizophrenia-related genes (eg NRG1, DTPN1) and genes in the myelination system (eg MBP, MOG, NRG1)

Genes repeatedly reported to be associated with the phenotype of interest should be included. In addition, databases being developed (see GEO and WebQTL in Electronic-Database Information) allow for retrieving and analyzing gene expression data according to researchers' particular interest, and may contribute to more extensive compilation of candidate genes in near future.

There are problems in this approach, though. First, manual literature mining on which compilation of candidate genes mostly depends is a tedious procedure. Secondly, there is no completely objective criterion to determine genes representing each hypothesis. Microarray studies may provide valid quantitative data on difference in expression level of each gene between bipolar and healthy subjects or between disease model and wild-type animals. However, such data may represent secondary effects of illness or treatment, or species-specific effects. Although a genetic association strategy can resolve this possibility, it will be a very small fraction of differentially expressed genes that directly affect susceptibility to the illness.

Despite these challenges, we have carried out compilation of a list of candidate genes pertinent to major hypotheses of major affective disorders (Table 2).

Neurotransmission systems

The monoamine (adrenergic, dopaminergic, and serotonergic) neurotransmission systems, which were the first to be hypothesized as systems whose derangements cause mood disorders, are offered here as a detailed example of candidate gene selection in the neurotransmission systems (see also Figure 1).

The genes for tyrosine hydroxylase (TH), which is a rate-limiting enzyme for dopamine and norepinephrine synthesis, and serotonin transporter (SLC6A4), which is a pump molecule for reuptake of synaptic serotonin into the presynaptic nerve terminal, have been among the most frequently studied candidates for affective disorders. Abnormalities of these genes can lead to decreased vesicular or synaptic monoamine levels as predicted by the original monoamine hypothesis. However, association has not been consistently replicated for any genes for synaptic components including TH and SLC6A4.²³ Genes for synaptic components of monoamine systems, nonetheless, still deserve genetic analysis, given the possible insufficiency of sample size and number of markers analyzed in the previous studies. In the presynaptic nerve terminal, these include genes for synthetic enzymes (eg TH, DBH, DDC), synaptic vesicle monoamine transporters (SLC18A1 and SLC18A2), and reuptake transporters (SLC6A2, SLC6A3, and SLC6A4), with many being shared between the three neurotransmitters (Table 2). Monoamines bind to pre- and postsynaptic receptors, of which numerous subtypes have been found so far. The list includes seven genes coding for adrenergic receptors, five for dopaminergic receptors, and 14 for serotonergic receptors, omitting those with limited roles in the brain such as beta-2-adrenergic receptor (ADRB2). Genes for catabolic enzymes bound to postsynaptic membrane (monoamine oxidases (MAOA and MAOB) and catechol-*O*-methyltransferase (COMT)) have also been included in the list.

Abnormality in postsynaptic signaling in bipolar disorder was first proposed in the phosphoinositide cycle because it is affected by lithium administration.^{24,25} Myo-inositol monophosphatase is inhibited

Table 2 Candidate genes pertinent to each putative pathological system: 1. Neurotransmission; 2. A neuroendocrine system; 3. Neurotrophic/growth factor systems; (1-3) Intracellular signaling largely shared by 1-3; 4. Circadian rhythm; 5. Genes implicated in the pathophysiology of other disease relevant to major affective disorders

Symbols	Genes	Aliases	Subcategories	Chromosomal region	Genomic size (bp)	References*
1. Neurotransmission system						
<i>Monoaminergic neurotransmission</i>						
ADRA1A	Alpha-1A-adrenergic receptor		Norepinephrine	8p21.2	117256	104
ADRA1B	Alpha-1B-adrenergic receptor		Norepinephrine	5q33.3	55762	
ADRA1D	Alpha-1D-adrenergic receptor		Norepinephrine	20p13	27844	104
ADRA2A	Alpha-2A-adrenergic receptor		Norepinephrine	10q25.2	3650	105, 106
ADRA2B	Alpha-2B-adrenergic receptor		Norepinephrine	2q11.2	3266	
ADRA2C	Alpha-2C-adrenergic receptor		Norepinephrine	4p16	2819	106, 107
ADRB1	Beta-1-adrenergic receptor		Norepinephrine	10q25.3	1714	108
QDPR	Quinoid dihydropteridine reductase		Metabolic enzyme	4p15.32	25691	
TPH1	Tryptophan hydroxylase 1		Metabolic enzyme	11p15.1	19772	109, 110
TPH2	Tryptophan hydroxylase 2		Metabolic enzyme	12q15	93595	111,112
HTR1A	5-Hydroxytryptamine (serotonin) receptor 1A		5-HT	5q12.3	1269	113
HTR1B	5-Hydroxytryptamine (serotonin) receptor 1B		5-HT	6q14.1	1173	114
HTR1D	5-Hydroxytryptamine (serotonin) receptor 1D		5-HT	1p36.12	2835	115
HTR1E	5-Hydroxytryptamine (serotonin) receptor 1E		5-HT	6q15	78988	
HTR1F	5-Hydroxytryptamine (serotonin) receptor 1F		5-HT	3q11.1	1101	
HTR2A	5-Hydroxytryptamine (serotonin) receptor 2A		5-HT	13q14.2	62661	113
HTR2B	5-Hydroxytryptamine (serotonin) receptor 2B		5-HT	2q37.1	15587	116
HTR2C	5-Hydroxytryptamine (serotonin) receptor 2C		5-HT	Xq23	326074	117
HTR3A	5-Hydroxytryptamine (serotonin) receptor 3A		5-HT	11q23.2	15195	118
HTR3B	5-Hydroxytryptamine (serotonin) receptor 3B		5-HT	11q23.2	41378	119
HTR4	5-Hydroxytryptamine (serotonin) receptor 4		5-HT	5q32	172618	120
HTR5A	5-Hydroxytryptamine (serotonin) receptor 5A		5-HT	7q36.2	13583	
HTR6	5-Hydroxytryptamine (serotonin) receptor 6		5-HT	1p36.13	14276	
HTR7	5-Hydroxytryptamine (serotonin) receptor 7		5-HT	10q23.31	115268	
SLC6A4	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	HTT SERT	5-HT	17q11.2	24118	121
ABCG1	ATP-binding cassette subfamily G member 1		5-HT	21q22.3	77974	122
DBH	Dopamine beta-hydroxylase precursor		Dopamine	9q34.2	22982	123
DRD1	D1 dopamine receptor		Dopamine	5q35.2	3127	124
DRD2	D2 dopamine receptor		Dopamine	11q23.2	65577	125
DRD3	D3 dopamine receptor		Dopamine	3q13.31	50200	126
DRD4	D4 dopamine receptor		Dopamine	11p15.5	3400	127
DRD5	D5 dopamine receptor		Dopamine	4p16.1	2032	
NR2A2	Nuclear receptor subfamily 4, group A, member 2	NURR1	Dopamine	2q24.1	8250	128
DDC	DOPA decarboxylase		Multiple monoaminergic systems	7p12.2	102610	129
MAOA	Monoamine oxidase A		Multiple monoaminergic systems	Xp11.3	70206	130
MAOB	Monoamine oxidase B		Multiple monoaminergic systems	Xp11.3	115765	

Table 2 Continued

Symbols	Genes	Aliases	Subcategories	Chromosomal region	Genomic size (bp)	References*
<u>TH</u>	Tyrosine hydroxylase		Multiple monoaminergic systems	11p15.5	7887	131, 132
<u>COMT</u>	Catechol-O-methyltransferase		Multiple monoaminergic systems	22q11	27 047	133
<u>SLC6A2</u>	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	NET	Multiple monoaminergic systems	16q12.2	46 031	134, 135
<u>SLC6A3</u>	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	DAT	Multiple monoaminergic systems	5p15.33	52 637	134, 135
<u>SLC18A1</u>	Solute carrier family 18 (vesicular monoamine), member 1	VMAT1	Multiple monoaminergic systems	8p21.3	38 346	64
<u>SLC18A2</u>	Solute carrier family 18 (vesicular monoamine), member 2	VMAT2	Multiple monoaminergic systems	10q26.11	36 203	136
<i>Cholinergic neurotransmission</i>						
<u>CHAT</u>	Choline acetyltransferase		Metabolic enzyme	10q11.23	56 010	137, 138
<u>CHRNA3</u>	Cholinergic receptor, nicotinic, alpha polypeptide 3		Receptor	15q24.3	25 679	137, 138
<u>CHRNA4</u>	Cholinergic receptor, nicotinic, alpha polypeptide 4		Receptor	20q13.33	16 298	137, 138
<u>CHRNA5</u>	Cholinergic receptor, nicotinic, alpha polypeptide 5		Receptor	15q24.3	27 806	137, 138
<u>CHRNA6</u>	Cholinergic receptor, nicotinic, alpha polypeptide 6		Receptor	8p11.21	15 857	137, 138
<u>CHRNA7</u>	Cholinergic receptor, nicotinic, alpha polypeptide 7		Receptor	15q13.3	184 762	137, 138
<u>CHRNB2</u>	Cholinergic receptor, nicotinic, beta polypeptide 2		Receptor	1q22	8827	137, 138
<u>CHRNB3</u>	Cholinergic receptor, nicotinic, beta polypeptide 3		Receptor	8p11.21	39 290	137, 138
<u>CHRM1</u>	Cholinergic receptor, muscarinic 1		Receptor	11q12.3	1383	139
<u>CHRM2</u>	Cholinergic receptor, muscarinic 2		Receptor	7q33	1401	44
<u>CHRM4</u>	Cholinergic receptor, muscarinic 4		Receptor	11p11.2	1455	44
<i>Amino-acid neurotransmission</i>						
<u>GABRA1</u>	Gamma-aminobutyric acid (GABA) A receptor, alpha 1		GABA	5q34	50 180	140
<u>GABRA2</u>	Gamma-aminobutyric acid A receptor, alpha 2		GABA	4p12	140 186	141-143
<u>GABRA3</u>	Gamma-aminobutyric acid A receptor, alpha 3		GABA	Xq28	283 210	141, 142
<u>GABRA5</u>	Gamma-aminobutyric acid A receptor, alpha 5		GABA	15q12	34 408	141, 142
<u>GABRB1</u>	Gamma-aminobutyric acid B receptor 1		GABA	6p22.1	30 856	144
<u>SLC6A1</u>	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1		GABA	9p25.3	21 553	145-147

<u>SLC6A11</u>	Solute carrier family 6 (neurotransmitter transporter, GABA), member 11	GAT1	GABA	Transporter	3p25.3	122230	145
<u>SLC6A12</u>	Solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12	BGT1	GABA	Transporter	12p13.33	23241	145
<u>VIAAT</u>	Vesicular inhibitory amino-acid transporter		GABA	Transporter	20q11.23	4887	148
<u>DBI</u>	Diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)		GABA	Others	2q14.2	4975	
<u>GAD2</u>	Glutamate decarboxylase 2	GAD65	Glutamate	Metabolic enzyme	10p12.1	87894	149
<u>GAD1</u>	Glutamate decarboxylase 1	GAD67	GABA	Metabolic enzyme	2q31.1	25848	149, 150
<u>ABAT</u>	4-Aminobutyrate aminotransferase precursor	GABA-T	Glutamate	Metabolic enzyme	16p13.2	109987	151
<u>GLRA3</u>	Glycine receptor, alpha 3		Glutamate	Receptor	4q34.1	186168	152
<u>GLRB</u>	Glycine receptor, beta		Glutamate	Receptor	4q32.1	95517	152
<u>GRIA1</u>	Glutamate receptor, ionotropic, AMPA 1		Glutamate	Receptor	5q33.2	364262	153
<u>GRIA2</u>	Glutamate receptor, ionotropic, AMPA 2		Glutamate	Receptor	4q32.1	143068	
<u>GRIA3</u>	Glutamate receptor, ionotropic, AMPA 3		Glutamate	Receptor	Xq25	304502	
<u>GRIA4</u>	Glutamate receptor, ionotropic		Glutamate	Receptor	11q22.3	368731	
<u>GRIK1</u>	Glutamate receptor, ionotropic kainate 1		Glutamate	Receptor	21q21.3	402421	
<u>GRIK2</u>	Glutamate receptor, ionotropic kainate 2		Glutamate	Receptor	6q16.3	669205	
<u>GRIK3</u>	Glutamate receptor, ionotropic kainate 3		Glutamate	Receptor	1p34.3	233113	
<u>GRIK4</u>	Glutamate receptor, ionotropic kainate 4		Glutamate	Receptor	11q23.3	325942	154
<u>GRIK5</u>	Glutamate receptor, ionotropic kainate 5		Glutamate	Receptor	19q32.2	64020	
<u>GRIN1</u>	N-methyl-D-aspartate receptor subunit zeta 1 (precursor)		Glutamate	Receptor	9q34.3	28919	155
<u>GRIN2A</u>	N-methyl-D-aspartate receptor subunit 2A		Glutamate	Receptor	16p13.2	421920	156
<u>GRIN2B</u>	N-methyl-D-aspartate receptor subunit 2B		Glutamate	Receptor	12q13.1	418909	
<u>GRIN2C</u>	N-methyl-D-aspartate receptor subunit 2C		Glutamate	Receptor	17q25.1	18802	
<u>GRIN2D</u>	N-methyl-D-aspartate receptor subunit 2D		Glutamate	Receptor	19q13.33	49262	157
<u>GRM1</u>	Glutamate receptor, metabotropic 1		Glutamate	Receptor	6q24.3	408316	
<u>GRM2</u>	Glutamate receptor, metabotropic 2		Glutamate	Receptor	3p21.31	9131	158
<u>GRM3</u>	Glutamate receptor, metabotropic 3		Glutamate	Receptor	7q21.12	220113	158
<u>GRM4</u>	Metabotropic glutamate receptor 4		Glutamate	Receptor	6p21.31	111816	
<u>GRM5</u>	Glutamate receptor, metabotropic 5		Glutamate	Receptor	11q14.3	540352	159
<u>GRM6</u>	Glutamate receptor, metabotropic 6		Glutamate	Receptor	5q35.3	16793	
<u>GRM7</u>	Glutamate receptor, metabotropic 7		Glutamate	Receptor	3p26.1	880272	160
<u>GRM8</u>	Metabotropic glutamate receptor 8		Glutamate	Receptor	7q31.33	804658	
<u>SLC1A1</u>	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	EAAT3	Glutamate	Transporter	9p24.2	96815	161-163
<u>SLC1A2</u>	Solute carrier family 1 (glial high-affinity glutamate transporter), member 2	EAAT2	Glutamate	Transporter	11p13	158150	161-163
<u>SLC1A3</u>	Solute carrier family 1 (glial high-affinity glutamate transporter), member 3	EAAT1	Glutamate	Transporter	5p13.2	81750	161-163
<u>SLC1A6</u>	Solute carrier family 1 (high-affinity aspartate/glutamate transporter), member 6	EAAT4	Glutamate	Transporter	19p13.12	22740	161, 163
<u>SLC6A9</u>	Solute carrier family 6 neurotransmitter transporter, glycine), member 9	GLYT1	Glutamate	Transporter	1p34.1	25587	164
<u>DAO</u>	D-amino-acid oxidase		Glutamate	Metabolic enzyme	12q24.11	20831	3
<u>SRR</u>	Serine racemase		Glutamate	Metabolic enzyme	17p13.3	21306	165
<i>Other neurotransmitter or neuromodulator systems</i>							
<u>AVP</u>	Arginine vasopressin-neurophysin II		Peptide	Transporter	20p13	2873	166
<u>AVPR1A</u>	Arginine vasopressin receptor 1A		Peptide	Receptor	12q14.2	6375	166

Table 2 Continued

<i>Symbols</i>	<i>Genes</i>	<i>Aliases</i>	<i>Subcategories</i>	<i>Chromosomal region</i>	<i>Genomic size (bp)</i>	<i>References*</i>
<u>CCK</u>	Cholecystokinin		Peptide	3p22.1	6802	
<u>CKAR</u>	Cholecystokinin A receptor		Peptide	4p15.2	9025	
<u>CKBR</u>	Cholecystokinin B receptor		Peptide	11p15.4	12202	167
<u>FCRT</u>	Orexin precursor		Peptide	17q21.2	1393	
<u>HCRTR1</u>	Orexin receptor 1		Peptide	1p35.2	8074	167
<u>HCRTR2</u>	Orexin receptor 2		Peptide	6p12.1	108347	167
<u>NPY</u>	Neuropeptide Y		Peptide	7p15.3	417	168
<u>NPY1R</u>	Neuropeptide Y receptor Y1		Peptide	4q32.2	2797	168
<u>NPY2R</u>	Neuropeptide Y receptor Y2		Peptide	4q32	1152	168
<u>NPY5R</u>	Neuropeptide Y receptor Y5		Peptide	4q32	4165	
<u>NTS</u>	Neurotensin		Peptide	12q21.31	8689	169
<u>NTSR1</u>	Neurotensin receptor 1		Peptide	20q13.33	53934	169
<u>NTSR2</u>	Neurotensin receptor 2		Peptide	2p25.1	12121	169
<u>SST</u>	Somatostatin		Peptide	3q27.3	1227	
<u>TAC1</u>	Tachykinin, precursor 1		Peptide	7q21.3	8408	
<u>TACR1</u>	Tachykinin receptor 1		Peptide	2p12	150044	
<u>TACR2</u>	Tachykinins receptor 2		Peptide	10q22.1	11498	
<u>TACR3</u>	Tachykinins receptor 3		Peptide	4q24	130349	170
<u>VIP</u>	Vasoactive intestinal peptide		Peptide	6q25.2	8857	
<u>VIPR2</u>	Vasoactive intestinal peptide receptor 2		Peptide	7q36.3	116783	66
<u>PMCH</u>	Pro-melanin-concentrating hormone	VPAC2	Peptide	12q23.2	1364	52
<u>GPR24</u>	G-protein-coupled receptor 24	MCHR1	Peptide	22q13.2	3582	52
<u>PDYN</u>	Beta-neoendorphin-dynorphin preproprotein		Peptide	20p13	15300	171
<u>OPRD1</u>	Opioid receptor, delta1		Opioid	1p35.3	51552	
<u>OPRK1</u>	Opioid receptor, kappa1		Opioid	8q11.23	21771	
<u>OPRM1</u>	Opioid receptor, kappa1		Opioid	6q25.2	80118	
<u>ADORA1</u>	Adenosine A1 receptor		Others	1q32.1	76750	172
<u>ADORA2A</u>	Adenosine A2a receptor		Others	22q11.23	9234	
<u>ADORA2B</u>	Adenosine A2b receptor		Others	17p12	30980	
<u>ADORA3</u>	Adenosine receptor A3		Others	1p13.2	4689	62

2. A neuroendocrine system

Gene	Protein	ACTH	Chromosomes	Gene	Protein	Chromosomes
<u>HFA axis</u>						
<u>POMC</u>	Proopiomelanocortin	ACTH	2q23.3		Neurotransmitter Intercellular signaling	7665
<u>CRH</u>	Corticotropin-releasing hormone precursor		8q13.1		Neurotransmitter Intercellular signaling	2080
<u>CRHR1</u>	Corticotropin-releasing hormone receptor 1		17q21.31		Receptor	51 525
<u>CRHR2</u>	Corticotropin-releasing hormone receptor 2		7p14.3		Receptor	29 697
<u>MC2R</u>	Melanocortin 2 receptor (adrenocorticotrophic hormone)		18p11.21		Receptor	894 173
<u>NR3C1</u>	Nuclear receptor subfamily 3, group C, member 1	(Glucocorticoid receptor)	5q31.3		Receptor	123 763 174
<u>NR3C2</u>	Nuclear receptor subfamily 3, group C, member 2	(Mineralocorticoid receptor)	4q31.23		Receptor	363 604 174
<u>MC4R</u>	Melanocortin 4 receptor		18q21.32		Receptor	999 52
<u>HSPA5</u>	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)	GRP78	9q33.3		Others	6478 175
<u>SERPINA6</u>	Corticosteroid binding globulin precursor	CBG	14q32.13		Others	19 088 55
<u>ABCB1</u>	ATP-binding cassette subfamily B member 1	ABCB1	7q21.12		Others	209 617 55
<u>HSD11B1</u>	11-Beta-hydroxysteroid dehydrogenase 1				Enzyme	48 746 56
<u>Neurotrophic/growth factor systems</u>						
<u>BDNF</u>	Brain-derived neurotrophic factor		11p14.1		Neurotransmitter Intercellular signaling	63 295 39, 60, 61
<u>EGF</u>	Epidermal growth factor		4q25		Neurotransmitter Intercellular signaling	99 370 176
<u>FGF2</u>	Fibroblast growth factor2		4q27		Neurotransmitter Intercellular signaling	71 528
<u>IGF1</u>	Insulin-like growth factor I		12q23.2		Neurotransmitter Intercellular signaling	84 649 62
<u>TGFB1</u>	Transforming growth factor, beta 1		19q13.2		Neurotransmitter Intercellular signaling	23561 177
<u>IGF1R</u>	Insulin-like growth factor 1 receptor precursor		15q26.3		Receptor	308 747 178
<u>NTRK2</u>	Neurotrophic tyrosine kinase, receptor, type 2	TrkB	9q21.33		Receptor	352 717 62
<u>NTRK3</u>	Neurotrophin receptor 3		15q25.3		Receptor	379 607
<u>(1-3) Intracellular signaling largely shared by 1-3</u>						
<u>ADCY2</u>	Adenylate cyclase 2		5p15.31		cAMP signaling	433 850 179
<u>ADCY9</u>	Adenylate cyclase 9		16p13.3		cAMP signaling	150 555 180
<u>ADRBK2</u>	Beta adrenergic receptor kinase 2		22q12.1		cAMP signaling	158 971 62
<u>CREB1</u>	CAMP-responsive element binding protein 1	GRK3	2q33.3		cAMP signaling	68 897 35, 179
<u>CREM</u>	CAMP-responsive element modulator		10p11.21		cAMP signaling	84 983 36, 179
<u>GNAI2</u>	Guanine nucleotide binding protein (G protein), alpha-activating activity polypeptide, olfactory type		3p21.31		cAMP signaling	22 633 33
<u>GNAL</u>	Guanine nucleotide binding protein (G protein), alpha-activating activity polypeptide, olfactory type		18p11.21		cAMP signaling	129 640 181

Table 2 Continued

Symbols	Genes	Aliases	Subcategories	Chromosomal region	Genomic size (bp)	References*
<u>GNAS</u>	Guanine nucleotide binding protein (G protein), alpha-stimulating activity polypeptide 1 (LL)		cAMP signaling	20q13.32	71451	33
<u>PDE4A</u>	Phosphodiesterase 4A, cAMP-specific		cAMP signaling	19p13.2	47837	37, 182
<u>PDE4B</u>	Phosphodiesterase 4B, cAMP-specific		cAMP signaling	1p31.2	580324	37, 183
<u>PDE4D</u>	Phosphodiesterase 4D, cAMP-specific		cAMP signaling	5q11.2	615565	184
<u>PRKACA</u>	Protein kinase, cAMP-dependent, catalytic, alpha		cAMP signaling	19p13.13	26045	185, 186
<u>PRKAR2B</u>	Protein kinase, cAMP-dependent, regulatory, type II, beta		cAMP signaling	7q22.3	116687	185
<u>RGS20</u>	Regulator of G-protein signaling 20		cAMP signaling	8q11.23	78299	187
<u>RGS4</u>	Regulator of G-protein signaling 4		cAMP signaling	1q23.3	5184	30, 73, 188
<u>RGS7</u>	Regulator of G-protein signaling 7		cAMP signaling	1q43	581608	179
<u>PPP1R1B</u>	Protein phosphatase 1, regulatory (inhibitor) subunit 1B	DARPP-32	cAMP signaling	17q12	9699	41
<u>PPP1R9B</u>	Protein phosphatase 1 regulatory subunit 9B	Spinophilin	Calcium signaling	17q21.33	15179	41, 189
<u>CAMK2A</u>	Calcium/calmodulin-dependent protein kinase II alpha		cAMP signaling	5q33.1	70277	39, 40, 190
<u>KCNK3</u>	Calcium-activated potassium channel SK3	hSK3	Calcium signaling	1q22	162714	191
<u>MARCKS</u>	Myristoylated alanine-rich protein kinase C		Calcium signaling	6q21	4423	192
<u>PRKCA</u>	Protein kinase C, alpha		Phosphoinositide	17q24.1	499979	192, 193
<u>PRKCE</u>	Protein kinase C, epsilon		Calcium signaling	2p21	532811	192, 193
<u>PLA2G1B</u>	Phospholipase A2, group 1B (paucas)		Calcium signaling	12q24.31	5674	194, 195
<u>PLCG1</u>	Phospholipase C, gamma 1		Phosphoinositide	20q12	38197	196
<u>GNB3</u>	Guanine nucleotide-binding protein beta-3		Neurotrophic factors	12p13.31	7183	197, 198
<u>BCL2</u>	B-cell CLL/lymphoma 2		Calcium signaling	18q21.33	195352	199
<u>DUSP6</u>	Dual-specificity phosphatase 6		cAMP signaling	12q21.33	4023	200
<u>MAP2K2</u>	Mitogen-activated protein kinase kinase 2		Phosphoinositide	19p13.3	33805	64, 199
<u>MAPK1</u>	Mitogen-activated protein kinase 1		Neurotrophic factors	22q11.21	105092	64, 193

<u>AKT1</u>	v-Akt murine thymoma viral oncogene homolog 1	Neurotrophic factors	14q32.33	23 856	42, 43
<u>GNAQ</u>	Guanine nucleotide binding protein (G protein), q	Phosphoinositide Phosphoinositide	9q21.2	311 000	201
<u>GNA11</u>	Guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	Phosphoinositide	19p13.3	26 923	201
<u>IMPA1</u>	Inositol(myo)-1(or 4)-monophosphatase 1	Phosphoinositide	8q21.13	28 365	26, 202
<u>IMPA2</u>	Inositol(myo)-1(or 4)-monophosphatase 2	Phosphoinositide	18p11.21	49 422	203
<u>INPP5F</u>	Inositol polyphosphate-5-phosphatase F	Phosphoinositide	10q26.12	103 044	203
<u>ITPKA</u>	1D-Myo-inositol-trisphosphate 3-kinase A	Phosphoinositide	15q15.1	9624	203
<u>ITPKB</u>	Inositol-1,4,5-trisphosphate-3 kinase B	Phosphoinositide	1q42.12	104 439	203
<u>PIB3PA</u>	Phosphatidylinositol (4,5) bisphosphate Phosphoinositide-3-kinase, class 2, beta polypeptide	Phosphoinositide	22q12.2	11 703	203
<u>PIK3C2B</u>	Phosphoinositide-3-kinase, class 2, beta polypeptide	Phosphoinositide	1q32.1	67 707	203
<u>PIK3C3</u>	Phosphoinositide-3-kinase, class 3	Phosphoinositide	18q12.3	126 246	203
<u>PIK4CA</u>	Phosphatidylinositol 4-kinase, catalytic, alpha peptide	Phosphoinositide	22q11.21	131 028	203
<u>PIF5K2A</u>	Phosphatidylinositol-4-phosphate 5-kinase type	Phosphoinositide	10p12.2	177 663	203
<u>KIAA0274</u>	Sac domain-containing inositol phosphatase 3	Phosphoinositide	6q21	134 167	203
<u>SYNJ1</u>	Synaptotagmin 1	Phosphoinositide	21q22.11	96 978	204
4. Circadian rhythm					
<i>Clock genes</i>					
<u>ARN1L</u>	Aryl hydrocarbon receptor nuclear translocator-like		11p15.3	109 433	66
<u>ARN1L2</u>	Transcription factor BMAL2		12p11.23	87 479	66
<u>BHLHB2</u>	Differentiated embryo chondrocyte expressed gene		3p26.1	5654	66
<u>aBHLHB3</u>	Basic helix-loop-helix domain containing, class B, 3		12p12.1	4886	66
<u>CLOCK</u>	Clock		4q12	114 338	66, 205
<u>CRY1</u>	Cryptochrome 1 (photolyase-like)		12q23.3	102 181	66
<u>CRY2</u>	Cryptochrome 2 (photolyase-like)		11p11.2	35 758	66
<u>CSNK1D</u>	Casein kinase 1, delta isoform 1		17q25.3	29 332	66
<u>CSNK1E</u>	Casein kinase 1 epsilon		22q13.1	25 461	66
<u>PER1</u>	Period 1		17p13.1	11 913	66
<u>PER2</u>	Period 2		2q37.3	44 407	66
<u>PER3</u>	Period 3		1p36.23	60 475	66
<u>TIMELESS</u>	Timeless (<i>Drosophila</i>) homolog		12q13.3	32 260	206
<u>DBP</u>	D site of albumin promoter (albumin D-box)		19q13.33	6642	66
<u>NR1D1</u>	Nuclear receptor subfamily 1, group D, member 1		17q21.1	7933	66
<i>Pathways for entrainment to light/darkness cycle and outputs of suprachiasmatic nucleus</i>					
<u>PROK2</u>	Prokineticin 2	Clock output	3p13	13 406	73
<u>GPR73L1</u>	G-protein-coupled receptor 73-like 1	Clock output	20p12.3	12 330	73
<u>TGFA</u>	Transforming growth factor alpha	Clock output	2p13.3	106 512	72
<u>ERBB1</u>	ERBB1	Clock output	7p11.2	137 918	72
<u>AANAT</u>	Arylalkylamine N-acetyltransferase	Melatonin	17q25.1	2549	
<u>MTNR1A</u>	Melatonin receptor 1A	Melatonin	4q35.2	22 675	207-209

Table 2 Continued

Symbols	Genes	Aliases	Subcategories	Chromosomal region	Genomic size (bp)	References*
<u>MTNR1B</u>	Melatonin receptor 1B		Melatonin	11q14.3	13 160	210, 208, 211
<u>CIRX</u>	Cone-rod homeobox		Clock output	19q13.33	5524	66
<u>OPN4</u>	Opsin4	Melanopsin	Photoreception	10q23.2	11 815	66
<u>ADCYAP1</u>	Adenylate cyclase-activating polypeptide	PACAP	Photoreception	18p11.32	5664	212, 213
<u>ADCYAP1R1</u>	Type 1 adenylate cyclase-activating polypeptide receptor		Photoreception	7p14.3	43 503	69, 212
<u>FYN</u>	Protein-tyrosine kinase fyn			6q21	212 143	66
<u>GDI1</u>	GDP dissociation inhibitor 1			Xq28	6293	66
<u>RAB3A</u>	RAB3A, member RAS oncogene family			19p13.11	7230	66
<u>NPAS2</u>	Neuronal PAS domain protein 2			2q11.2	175 551	214
5. Genes implicated in pathophysiology of other diseases relevant to major affective disorders						
<i>Genes implicated in the pathophysiology of Parkinson's disease</i>						
<u>PARK2</u>	Parkinson disease (autosomal recessive, juvenile) 2, parkin	PARK2		6q26	1 379 130	76
<u>SNCA</u>	Synuclein, alpha	PARKIN		4q22.1	111 429	76
<u>PNUTL1</u>	Peanut-like 1	PARK1		22q11.21	8818	76
<u>SNCAIP</u>	Synuclein alpha-interacting protein	CDCREL1		5q23.2	151 817	76
<u>UCHL1</u>	Ubiquitin carboxyl-terminal esterase L1	Synphilin1		4p13	11 518	76
<u>GPR37</u>	G-protein-coupled receptor 37	PARK5		7q31.33	19 566	76
<u>UBB</u>	Ubiquitin B precursor	PAELR		17p11.2	1688	76
<u>UBE1</u>	Ubiquitin-activating enzyme E1			Xp11.3	24 267	76
<u>STUB1</u>	STP1 homology and U-box containing protein 1	CHIP		16p13.3	2277	76, 79
<u>UBE2L3</u>	Ubiquitin-conjugating enzyme E2L 3	UBCH7		22q11.21	56 367	76
<u>UBE2L6</u>	Ubiquitin-conjugating enzyme E2L 6	UBCH8		11q12.1	16 325	76
<u>PARK7</u>	Parkinson disease (autosomal recessive, early onset) 7 (PARK7)	DJ1		1p36.23	23 544	215
<i>Genes implicated in the pathophysiology of schizophrenia</i>						
<u>RELN</u>	Reelin		Cell migration	7q22.1	517 727	150
<u>DISC1</u>	Disrupted in schizophrenia 1		Cell migration	1q42.2	399 739	93, 216, 217
<u>NDEL1</u>	nucleolar distribution gene E homolog like 1	NUDEL	Cell migration	17p13.1	32 293	217
<u>PAFAH1B1</u>	Platelet-activating factor acetylhydrolase, isoform 1b, alpha subunit 45 kDa	LIS1	Cell migration	17p13.3	91 953	217, 218
<u>PTAFR</u>	Platelet-activating factor receptor		Cell migration	1p35.3	27 328	219
<u>CHL1</u>	Cell adhesion molecule with homology to L1CAM		Cell migration	3p26.3	212 449	220
<u>L1CAM</u>	L1 cell adhesion molecule isoform 1 precursor		Cell migration	Xq28	13 925	221, 222
<u>NCAM1</u>	Neuronal cell adhesion molecule		Cell migration	11q23.1	314 048	66, 223
<u>DTNBP1</u>	Dysbindin1		Schizophrenia gene	6p22.3	140 167	1
<u>NRG1</u>	Neuregulin1		Schizophrenia gene	8p12	1 103 459	2
<u>PRODH</u>	Prolin dehydrogenase (oxidase) 1		Schizophrenia gene	22q11	23 771	224
<u>CLDN11</u>	Oligodendrocyte transmembrane protein		Myelination	3q26.2	13 827	94

ERBB3	v-Erb-b2 erythroblastic leukemia viral oncogene	Myelination	12q13.2	22 605	94
GALC	Galactosylceramidase precursor	Myelination	14q31.3	60 484	94
MBP	Myelin basic protein	Myelination	18q23	37 252	94
MOG	Myelin oligodendrocyte glycoprotein	Myelination	6p22.1	15 621	94
OLIG1	Oligodendrocyte transcription factor 1	Myelination	21q22.11	2276	94
OLIG2	Oligodendrocyte lineage transcription factor 2	Myelination	21q22.11	3209	94
PLP1	Proteolipid protein 1	Myelination	Xq22.2	15 706	94
SOX10	SRY (sex determining region Y)-box 10	Myelination	22q13.1	12 220	94
TF	Transferrin	Myelination	3q22.1	32 401	94

The total number of candidate genes: 257; total genomic size: 26 620 628 bp.

^aWhen a gene has not intensively been studied despite its putative key biologic role in one of the pathways, it may lack a reference. Conversely, when numerous studies have been conducted, review articles, meta-analyses, and large-scale studies are preferentially given.

by lithium,^{24,25} and its coding gene (IMPA2) is a promising candidate.^{26,27} Another gene in this pathway, phosphoinositide-3-kinase class 3 (PIK3C3), was recently reported to be associated with bipolar disorder and schizophrenia.²⁸ Calcium signaling is closely linked to the phosphoinositide pathway, and expression of protein kinase C subtypes (PRKCA and PRKCE) and its substrate myristoylated alanine-rich protein kinase C (MARCKS) are reduced in the rat brain after chronic treatment with lithium.²⁹

Monoamine neurotransmitter receptors, such as alpha2 and beta-1-adrenergic receptors, are coupled to G proteins, which, upon stimulation, activate enzymes in the cAMP-signaling pathway. The gene for regulator of G-protein signaling 4 (RGS4) was initially brought into attention by a microarray study and recently reported to be associated with schizophrenia.³⁰ Selecting candidates based on expression data also led to the detection of associations of G-protein-coupled receptor kinase3 (GRK3)³¹ and other promising gene³² with bipolar disorder. Altered expression level of G protein AS and A12 subunits (GNAS, GNAI2) in the post-mortem brains from bipolar or lithium receiving subjects has also been reported,³³ although variants in the former gene are not apparently associated with bipolar disorder.³⁴ Recent animal studies demonstrated that chronic administration of antidepressants induces elevation of cAMP-responsive element binding protein gene (CREB1) expression³⁵ and cAMP-responsive element modulator (CREM)-deficient mice showed emotional and behavioral changes.³⁶ Also, chronic antidepressant administration increases cAMP phosphodiesterase (PDE4A and PDE4B) expression in rat frontal cortex.³⁷ A phosphodiesterase inhibitor, rolipram, has been reported to have an antidepressive effect.³⁸

Further, abnormalities of molecules that overarch multiple intracellular signaling pathways (eg calcium/calmodulin-dependent protein kinase II alpha (CAMK2A),^{39,40} DARPP-32 (PPP1R1B),⁴¹ and v-akt murine thymoma viral oncogene homologs (AKT1),^{42,43} are also suggested in psychiatric illnesses.

Roles of other neurotransmission systems including cholinergic, amino acid (glutamate and GABA) and peptidergic neurotransmission in bipolar disorder or related physiological functions such as appetite and anxiety are also supported by neuropharmacological findings,⁴⁴⁻⁴⁸ although not detailed here.

A neuroendocrine system

The hypothalamic-pituitary-adrenocortical (HPA) axis has a long history as a stress-response pathway and has been repeatedly suggested to play a role in major depressive disorder.⁴⁹ A recent hypothesis that elevated levels of cortisol in depressed patients may contribute to neuronal death and to reduced dendritic arborizations in hippocampus⁵⁰⁻⁵² seems to have a potential for elucidating the etiology of mood disorder. This hypothesis is consistent with the

previous neuroimaging findings reporting reductions of hippocampal volume in some mood disorder subjects.^{53,54}

Obvious candidates based on these formulations are genes encoding peptide hormones (proopiomelanocortin (POMC) and corticotrophin-releasing hormone precursor (CRH)) and their receptors (MC2R, MC4R, CRHR1, and CRHR2). In addition, glucocorticoid receptor (NR3C1), which binds to glucocorticoids and then enters the nucleus to enhance or inhibit gene expression by direct binding to glucocorticoid response elements or by interactions with other transcriptional factors such as CREB, can be considered an important signaling component. Other candidates include genes for heat-shock proteins such as HSPA5, which associate with the glucocorticoid receptor as chaperones, multidrug-resistant protein 1 (ABCB1), which pumps out cortisol from the cell,⁵⁵ and 11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1), which metabolizes cortisol.⁵⁶

Neurotrophic factor systems

There has been growing evidence supporting roles of neurotrophic factors and growth factors, which regulate neuronal growth, development, survival, and plasticity, in mood disorders. The gene for brain-derived neurotrophic factor (BDNF), which is involved in neuronal survival and arborization in hippocampus, is an unusually promising candidate. The expression of BDNF is decreased by stress and glucocorticoids⁵⁷ and is increased by chronic antidepressant or electroconvulsive treatment in rat hippocampus.^{58,59} Association between BDNF and bipolar disorder has been replicated in independent pedigree samples.^{60,61} Also, the gene for insulin-like growth factor I (IGF1) would be worth studying based on its role in neurogenesis and reported altered expression level in the brains of amphetamine-treated rats.⁶² Recently, a requirement was demonstrated for hippocampal neurogenesis for behavioral effects of antidepressants, consistent with importance of genes involved in neurogenesis in studies of depression.⁶³

As in the neurotransmission systems and HPA axis, genes for molecular components of the neurotrophic factor system have not been systematically studied for association with bipolar disorder. Genes such as NTRK2 and NTRK3 coding for neurotrophic factor receptors collectively called Trk are candidates as well as genes for ligands. Among the several intracellular cascade systems activated upon Trk stimulation are phosphoinositide signaling and protein kinase C pathway, whose components are shared with neurotransmission systems described above. It may be challenging to select candidates from the mitogen-activated protein (MAP) kinase cascade, another intracellular signaling pathway downstream of Trk, because of the large number of subtypes for each protein. However, an expression study on PC12 cells differentiated by nerve growth factor (NGF)

showed that lithium administration altered expression of two genes (MAP2K2 and MAPK1) encoding kinases of this pathway.⁶⁴

Circadian rhythm

Abnormalities in circadian rhythm are found in seasonal affective disorder as well as in a fraction of patients with major depression. The fact that interventions on circadian rhythm such as light therapy and sleep deprivation can improve the symptoms of depression or provoke mania might be indicative of an etiological role of this system.⁶⁵ The mammalian circadian pacemaker is located within the suprachiasmatic nucleus (SCN) in the hypothalamus. 'Clock genes' (eg CLOCK, ARNTL (BMAL1), ARNTL2 (BMAL2), PER1, PER2, and PER3) play crucial roles in generating and regulating circadian rhythm, and mutations of these genes have already been reported to cause abnormal circadian locomotion in rodents. In the past several years, many clock genes have been identified in various species such as *Drosophila*, fungi, and rodents. As human counterparts or homologues have already described for most clock genes,⁶⁶ the circadian rhythm system is amenable to genetic dissection.

Although the clock genes are probably the first candidates to be studied in the circadian rhythm system, it is noteworthy that these gene loci did not show major effect on strain variability in mouse circadian behavior in a genomewide analysis.⁶⁷ Studying nonclock genes with suggested roles in circadian rhythm would also be important. Since the SCN can be entrained to light/dark cycle, genes encoding components involved in photoreception in the retinohypothalamic tract are intriguing candidates. For example, pituitary adenylate cyclase-activating polypeptide (ADCYAP1) is a major neurotransmitter of this tract as well as glutamate.⁶⁸ Lack of either of its receptor genes, ADCYAP1R1 or VIPR2, leads to abnormal circadian phenotype in rodents.⁶⁹ In addition, since diurnal rhythmicity in physiological functions and behaviors is eventually affected in mood disorder, output pathways from the SCN, including pineal melatonin secretion,^{70,71} cannot be omitted. Recently, transforming growth factor- α (TGFA)⁷² and prokineticin 2 (PROK2),⁷³ substances secreted from the SCN to adjacent hypothalamic areas, have been reported to regulate behavioral circadian rhythm in mice.

Systems implicated in Parkinson's disease and schizophrenia

Other CNS diseases may also provide clues for susceptibility genes for mood disorders, if they share etiological mechanisms with mood disorders. Symptoms of depression occur in approximately half of the subjects with Parkinson's disease. Although neuropathological changes characteristic of Parkinson's

disease such as Lewy body formation and demise of dopaminergic neurons in the substantia nigra are not generally observed in the post-mortem brains of mood disorder subjects, depression is reported to be a risk factor for developing Parkinson's disease,^{74,75} suggesting a mechanism shared in part by both illnesses. Recent studies have revealed that genes playing roles in the ubiquitin-proteasome pathway cause some familial forms of Parkinson's disease (SNCA, PARK2 (PARKIN), UCHL1).⁷⁶ Several other components that play crucial roles in this pathway have also been reported; for example, Parkin-associated endothelin receptor-like receptor (Pael-R)⁷⁷ and CDCrel-1 (PNU TL1)⁷⁸ suggested for one of the substrates for PARKIN-mediated ubiquitination. Also, a protein called carboxy-terminus of Hsp70p-interacting protein (CHIP) is known to modulate the function of PARKIN.⁷⁹

Also, the hypothesis that one subclass of major affective disorders susceptibility genes in common with schizophrenia is particularly promising. Genetic epidemiology has provided evidence for this overlap, primarily in family studies. Gershon *et al* observed an excess of major depression and schizoaffective disorder in the relatives of both mood disorder and schizophrenia probands.^{80,81} The excess of major depression in relatives of both mood disorders and schizophrenia has been a consistent finding.⁸² Studies from three data sets have addressed the issue of psychotic mood disorder/schizophrenia overlap. Two of the data sets found elevated rates of psychotic mood disorder in relatives of schizophrenic probands, and *vice versa*;^{83–86} the third also suggested shared liability.⁸⁷ In addition, some twin studies have found evidence of shared heritability between psychotic mood disorder and schizophrenia.^{88,89} Further, linkage studies of bipolar illness and schizophrenia have implicated overlapping chromosomal regions, including 10p12–13, 13q31–33, 18p11.2, and 22q11–13,^{90,91} although not all analyses agree.⁹² There has been considerable progress in identifying genes associated with schizophrenia, particularly in chromosomal regions where evidence of linkage was suggested. Among them, the G72/G30 gene locus on 13q33 has been demonstrated to be associated with both schizophrenia and bipolar disorder.^{3,4,6–8} The notion of shared susceptibility gene is also supported by a very recent association study on DISC1 gene.⁹³ Other schizophrenia genes such as NRG1 and DTNBP might also be worth studying for possible association with mood disorders. In addition, a recent study has demonstrated convergent expression alterations of genes involved in myelination in both schizophrenia and bipolar disorder.⁹⁴ Such genes would be good candidates for susceptibility genes shared by major psychiatric illnesses. Studying these genes implicated in the pathophysiology of schizophrenia may contribute to eventual reconstruction of the current diagnostic nosology, and to identification of new molecular targets with broad therapeutic spectra.

Prioritizing candidate genes by quantitative trait loci (QTL) analysis

Combining microarray gene expression data and gene mapping methods to identify genetic determinants of gene expression (expression phenotypes) has recently been applied in several species, including mouse and human.^{95–97} This has resulted in the successful identification of QTLs, which control the baseline expression levels of some genes. We have used this approach to identify regulators of the expression of the candidate genes we compiled, in the adult BXD recombinant inbred mice. We decided to use QTL mapping data in mouse instead of human, because the only available human QTL mapping results are from lymphoblast cell lines, and it has been shown in mouse that QTLs in brain and hematopoietic stem cells differ greatly.⁹⁸ Interval mappings were performed at the WebQTL site (see Electronic-Database Information), using UTHSC Brain mRNA U74Av2 (Mar04) RMA Orig database. QTL with an empirical genome-wide *P*-value less than 0.05 was detected for six genes, namely HTR2B, HTR4, GRIN2B, PRKCE, PER3, and BCL2.

We then determined if any of the QTLs is in syntenic regions to human bipolar linkage findings. If a *cis*-acting QTL, for which the QTL is in the target gene itself, overlaps with bipolar linkage, the target gene itself merits testing in association studies as positional candidate for bipolar linkage. If the overlapping QTL is a *trans*-acting QTL, the regulator at the QTL is a new candidate gene for association study. Thus, linkage results to gene expression may point to new candidate genes and underlying regulatory pathways for the bipolar linkage. We found that two QTLs overlap with bipolar linkage regions. A *trans*-linked QTL for two genes, HTR4 and BCL2, is mapped to the same region in mouse genome, and may thus represent a single linkage. This *trans*-linked QTL for the two genes can be divided into four segments, three of which are in syntenic regions to bipolar linkage findings at 2q,⁹² 6q,⁹⁹ and 10q,⁹² respectively. In addition, a *cis*-QTL for the gene PER3 is in syntenic region to bipolar linkage finding at 1p.⁹² This suggests that PER3 is a good candidate for this bipolar linkage. With the identification of more bipolar linkages and the improvement of QTL mapping methods, the list of genes with QTLs overlapping with bipolar linkage will certainly grow.

Requirements for implementation of the systems genetic approach and future directions

The approach being suggested would benefit from the feasibility of much denser genotyping compared to the whole-genome LD mapping. It requires collection of information on functional importance of polymorphic markers, as well as positions, flanking sequences, validation status, and allele frequencies. Since the information is scattered on multiple web-based databases such as those from UCSC Genome

Bioinformatics, dbSNP, HapMap, and SNP Consortium (see Electronic-Database Information), manual mining of information can be tedious and sometimes infeasible. What is needed is a sophisticated informatics system facilitating compilation of pieces of information from different resources into a single platform. We might further assign priority of genotyping to each polymorphism according to its potential functional effect and the degree of LD with other polymorphisms.

Genotype data obtained by the study of multiple genes in a biologic system may provide a set of multiple susceptibility genes either through conventional association analyses or through multilocus association analyses such as the one developed by Hoh *et al.*²¹ Although the latter may provide a list of susceptibility genes, in which some of them are exerting interacting effects, we further need computational modeling, which allows for systems analysis describing specific relationships between genes and clinical features. This would provide a basis for putting genetic results back into biological and clinical context. The systems listed in Table 1 are considered more complex in reality than described above, and it is also possible that interactions between systems rather than within a system increase the risk for major affective disorders. For example, a suggested integral model views multiple systems from a single perspective of neuronal death/survival. Hyperfunction of glutamatergic neurotransmission and HPA axis can lead to neuronal death, whereas adrenergic/serotonergic neurotransmission and neurotrophic factors favor neuronal survival/arborization or neurogenesis, with each system interacting with several others.^{50–52} The hypothesis-based study described so far is expected to increase the likelihood of obtaining outputs that can be reasonably interpreted through the current biological and epidemiological knowledge of major affective disorders. The systems functioning conclusions from the genetic outputs, although, would not necessarily be completely consistent with the current hypothesis-based systems. The biological meaning of the genetic outputs could be tested by further research designs such as multiple gene manipulations in rodents.

Electronic-Database Information

Databases for biologic pathways

Gene Ontology (GO) Consortium:

<http://www.geneontology.org/>

Kyoto Encyclopedia of Genes and Genomes (KEGG) databases:

<http://www.genome.ad.jp/kegg/pathway.html>

Databases for genomic information and gene expression

UCSC Genome Bioinformatics:

<http://genome.ucsc.edu/>

dbSNP: <http://www.ncbi.nlm.nih.gov/SNP/>

The International HapMap Project:

<http://www.hapmap.org/>

The SNP Consortium: <http://snp.cshl.org/>

Gene Expression Omnibus (GEO):

<http://www.ncbi.nlm.nih.gov/geo/>

WebQTL: <http://www.genenetwork.org/>

Candidate gene projects involving resequencing

The NIEHS SNPs program:

<http://egg.gs.washington.edu/>

The Cardiogenomics program:

<http://www.cardiogenomics.org>

The SeattleSNPs program:

<http://pga.gs.washington.edu/>

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