

Discovering Endophenotypes for Major Depression

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The limited success of genetic studies of major depression has raised questions concerning the definition of genetically relevant phenotypes. This paper presents strategies to improve the phenotypic definition of major depression by proposing endophenotypes at two levels: First, dissecting the depressive phenotype into key components results in narrow definitions of putative psychopathological endophenotypes: mood bias toward negative emotions, impaired reward function, impaired learning and memory, neurovegetative signs, impaired diurnal variation, impaired executive cognitive function, psychomotor change, and increased stress sensitivity. A review of the recent literature on neurobiological and genetic findings associated with these components is given. Second, the most consistent heritable biological markers of major depression are proposed as biological endophenotypes for genetic studies: REM sleep abnormalities, functional and structural brain abnormalities, dysfunctions in serotonergic, catecholaminergic, hypothalamic-pituitary-adrenocortical axis, and CRH systems, and intracellular signal transduction endophenotypes. The associations among the psychopathological and biological endophenotypes are discussed with respect to specificity, temporal stability, heritability, familiarity, and clinical and biological plausibility. Finally, the case is made for the development of a new classification system in order to reduce the heterogeneity of depression representing a major impediment to elucidating the genetic and neurobiological basis of this common, severe, and often life-threatening illness.

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INTRODUCTION

Scientific advances suggest that the time is at hand to begin to elucidate the genetic basis of mood disorders: (1) detection of genes for brain diseases such as Huntington's disease and Alzheimer's disease; (2) dramatic developments in molecular genetics including the human genome project and increasing availability of genetic markers throughout the genome; and (3) consistent evidence from twin and family studies that genes are substantially involved in the susceptibility to mood disorders. However, despite costly candidate gene association studies and genome-wide linkage scans, no genes for major depression have been consistently identified. Fortunately, in certain families, there is preliminary evidence that recurrent, early-onset major depression is linked to a region containing the CREB1 gene. This gene constitutes an attractive candidate

as a susceptibility allele for major depression because CREB1 plays major roles in neuronal plasticity, cognition, and memory (Zubenko *et al*, 2002).

The limited success of genetic studies of complex disorders has resulted in a considerable debate regarding the reasons for the failure in the past, and the best methodological approach to take in the future. Questions have been raised concerning both the definition of genetically relevant phenotypes and the number and nature of the underlying genes.

The use of the current classification schemas including DSM-IV undoubtedly contributes to the difficulties in finding genes for psychiatric disorders. They are based on clusters of symptoms and characteristics of clinical course that do not necessarily describe homogenous disorders, and rather reflect common final pathways of different pathophysiological processes (Charney *et al*, 2002). Moreover, it has been argued that a continuum of depressive symptoms exists from normal to pathological, and that clinical symptomatology does not point to a simple categorical classification (Angst and Dobler-Mikola, 1984; Angst and Merikangas, 2001). Finally, mood and anxiety disorders as defined by the DSM show high comorbidity and substantial symptomatic fluidity with frequent changes of diagnostic subtypes over time (Angst and Dobler-Mikola, 1985; Angst *et al*, 1990, 2000; Merikangas *et al*, 2003).

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Moreover, genes and behaviors may not be associated on a simplistic, one-to-one basis; the true relationship between a gene and a behavior is probably more akin to chaos theory's 'sensitive dependence on initial conditions'. For example, there is presumably no gene for 'language'; there are a number of genes that pattern the embryonic brain in such a way as to facilitate, and allow the physiological processes necessary for, language acquisition. In a similar manner, no gene has been found to singularly code for a human psychiatric condition. Indeed, it is unlikely that any single gene does code for a psychiatric condition *per se*, but rather that susceptibility genes interacting with developmental factors, both day-to-day and profound environmental events, epigenetic DNA modifications, and possible entirely stochastic mechanisms eventually lead to the development of normal and abnormal human behaviors (Petronis, 2001; Glazier et al, 2002).

In this paper, we will present strategies to overcome the methodological difficulties mentioned above with respect to the elucidation of the genetic basis of major depressive disorder (MDD) by proposing putative endophenotypes. The term 'endophenotype' was described as an internal phenotype (ie not obvious to the unaided eye) that fills the gap between available descriptors and between the gene and the elusive disease process (Gottesman and Shields, 1973), and therefore may help to resolve questions about etiological models. The endophenotype concept was based on the assumption that the number of genes involved in the variations of endophenotypes representing relatively straightforward and putatively more elementary phenomena (as opposed to behavioral macros) are fewer than those involved in producing a psychiatric diagnostic entity (Gottesman and Gould, 2003). Endophenotypes provide a means for identifying the 'downstream' traits of clinical phenotypes, as well as the 'upstream' consequence of genes. The methods available to identify endophenotypes include neuropsychological, cognitive, neurophysiological, neuroanatomical, and biochemical measures (Figures 1 and 2). The evaluation of endophenotypes is based on the following criteria (Tsuang et al, 1993):

Specificity: The endophenotype is more strongly associated with the disease of interest than with other psychiatric conditions.

State-independence: The endophenotype is stable over time and not an epiphenomenon of the illness or its treatment.

Heritability: Variance in the endophenotype is associated with genetic variance.

Familial association: The endophenotype is more prevalent among the relatives of ill probands compared with an appropriate control group.

Cosegregation: The endophenotype is more prevalent among the ill relatives of ill probands compared with the well relatives of the ill probands.

Biological and clinical plausibility: The endophenotype bears some conceptual relationship to the disease.

We will present and discuss endophenotypes at two levels: First, we will dissect the DSM-IV phenotype into narrow psychopathological characteristics that are biologically and clinically meaningful and can be assessed quantitatively. We will refer to these core psychopathological features of major depression as *key components*; we will refer to those key

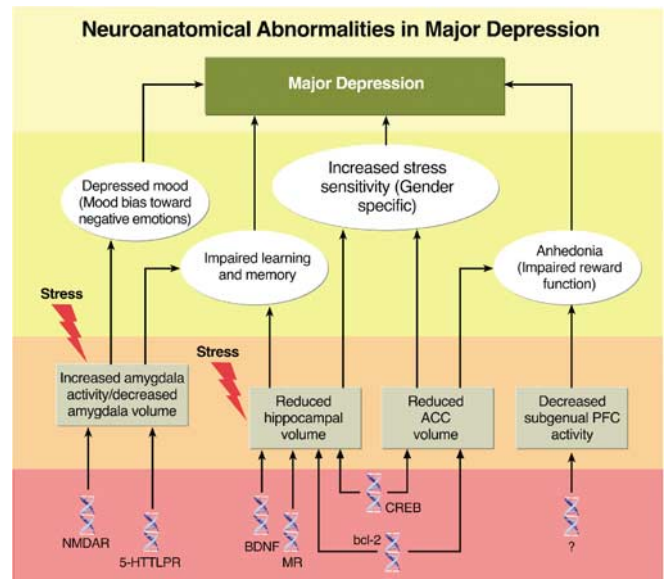


Figure 1 Example of how neuroanatomical abnormalities may relate to candidate genes and to key components of major depression. Some of the key components have a greater potential to serve as endophenotypes than others (see Table 1). Not all functional directions are indicated for the purpose of clarity of the figure.

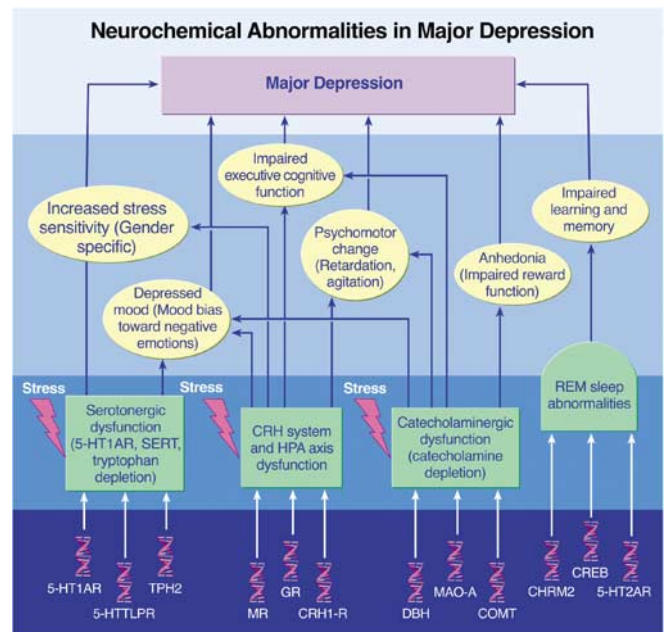


Figure 2 Example of how neurochemical abnormalities may relate to candidate genes and to key components of major depression. Some of the key components have a greater potential to serve as endophenotypes than others (see Table 1). Not all functional directions are indicated for the purpose of clarity of the figure.

components that meet some of the endophenotype criteria as *psychopathological endophenotypes*. Second, we present biological markers that are useful for genetic studies. We will refer to biological markers that meet some of the endophenotype criteria as *biological endophenotypes*. All endophenotypes presented in this paper have to be seen as

putative endophenotypes because whether or not they consistently meet all required endophenotype criteria has not been determined yet. We will review the literature and indicate the strength and limitations of psychopathological and biological endophenotypes in MDD with a special emphasis on studies associating endophenotypes with genes. Finally, we present future directions for the elucidation of the genetic basis of MDD.

KEY COMPONENTS OF MAJOR DEPRESSION

The validity of symptoms and components of MDD has been difficult to achieve because the MDD criteria likely encompass a group of disorders that are heterogeneous with respect to etiology and pathophysiology. Based on factor and cluster analytic studies, Nelson and Charney (1981) found that mood bias toward negative emotions, anhedonia, and psychomotor symptoms best characterize MDD. However, the diagnostic criteria for a disorder based on clinical characteristics without knowledge of the underlying etiological processes cannot be validated adequately. The use of more indirect indicators of disease validity or validity of depressive subtypes such as family history, treatment response, longitudinal course and stability, patterns of comorbidity, and social consequences led to circularity in validating the criteria (Kendell, 1989). Not surprisingly, studies on the biological basis of depression have found stronger associations between specific biological dysfunctions and certain components of major depression than with the presence or absence of DSM-IV MDD: symptoms such as cognitive deficits, rumination, psychomotor retardation, anhedonia, and lowered mood have been associated with specific focal abnormalities of regional cerebral blood flow (CBF; Mayberg *et al*, 1999; Drevets, 2000). Moreover, components of major depression such as altered response to stress (Caspi *et al*, 2003), impaired cognitive abilities, and dysfunctional reward-related behaviors are easier to model in animals than the depressive syndrome itself (Redei *et al*, 2001). Finally, intermediate levels of recurrence of depressive episodes have been associated with high genetic liability of MDD (Kendler *et al*, 1999), while a high temporal stability of the phenotype is favorable for genetic studies. The ensuing discussion dissects MDD into its key components based on biological validity, availability of accurate and quantitative assessment methods, and clinical relevance.

Depressed Mood (Mood Bias Toward Negative Emotions)

Depressed mood is a core symptom of MDD (Nelson and Charney, 1981). Attentional and mnemonic biases toward processing of mood-congruent information including sad, unpleasant and negative words, emotional facial expressions, and memories also are reliable and relatively specific neuropsychological findings of MDD (Watkins *et al*, 1996; Murphy *et al*, 1999), and have been found in remitted depressed subjects suggesting some state-independence (Hammen *et al*, 1985; Koschack *et al*, 2003). Experimental depletion of central serotonin led to the emergence of mood-congruent memory bias (Klaassen *et al*, 2002), and

physiological activation of the brain regions that have been found to be involved in mood-congruent information processing (Elliott *et al*, 2002) is thought to play a key role in MDD (Drevets *et al*, 1997), thus implicating good biological validity. Attentional and mnemonic biases in depression have high clinical relevance because they are related to cognitive-behavioral theories of depression, upon which treatment strategies have been based (Beck, 1967). However, there is only preliminary data on the heritability of mood bias: recurrent thoughts of death and suicide appeared to be a specific characteristic of familial MDD (Kendler *et al*, 1999).

Anhedonia (Impaired Reward Function)

Loss of interest, lack of reactivity, and anhedonia also constitute core features of major depression (Nelson and Charney, 1981), and these symptoms represent key diagnostic criteria for the DSM-IV melancholic subtype of major depression. Anhedonia seems to be a relatively specific feature of depression (Fawcett *et al*, 1983), and even in patients with schizophrenia, anhedonia has been related to the depressive syndrome rather than to the deficit syndrome (Loas *et al*, 1999). Associations between dysfunctions of the brain reward system and anhedonia are the basis of the biological plausibility of anhedonia-related endophenotypes. Specifically, neurotrophic factors including cAMP response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) and the transcription factor delta-FosB may represent molecular mechanisms involved in long-term alterations of the brain reward system (Nestler *et al*, 2001, 2002). Enhanced rewarding effects of dextroamphetamine found in patients with MDD may represent hypofunction of the dopaminergic system associated with anhedonia (Tremblay *et al*, 2002). Preliminary evidence for the potential heritability of these findings includes a functional polymorphism of the catechol-*O*-methyltransferase (COMT) gene that has been associated with the individual variation in the brain response to dopaminergic challenge (Mattay *et al*, 2003).

Epidemiological research provides clues for state-independence, heritability, and familial association of dysfunctions of the brain reward system as endophenotype for MDD. For example, anhedonia symptoms often precede the onset of MDD (Dryman and Eaton, 1991) and seem to be relatively stable over time (Oquendo *et al*, 2004). A sib-pair study showed that the personality construct 'reward dependence' has trait-like characteristics related to the familiarity of major depression (Farmer *et al*, 2003). Given the extensive evidence for associations between impairments of brain reward pathways and addiction (Nestler *et al*, 2002), familial coaggregation and lifetime comorbidity of substance use disorders and MDD (Kendler *et al*, 1997; Brook *et al*, 2002) also suggest persistent familial abnormalities of brain reward pathways in MDD.

Impaired Learning and Memory

Diminished ability to attend or concentrate is a diagnostic criterion of DSM-IV major depressive episode, which reflects cognitive impairments including learning and long-term memory, and deficits in working memory

(short-term memory) and selective attention (Burt *et al*, 1995; Landro *et al*, 2001). These impairments are not specific for depression, although the pathophysiological mechanisms underlying these features of depression may differ from those in other psychiatric disorders (Berman *et al*, 1993; Barch *et al*, 2003). Specifically, abnormal reduction of CBF and metabolism in the dorsolateral prefrontal cortex and dorsal anterior cingulate cortex (ACC) might explain diminished ability to attend and concentrate in MDD (Drevets and Raichle, 1998), thus providing some evidence for the biological plausibility of this putative endophenotype for MDD.

Impaired concentration and attention were found to be frequent and early prodromal signs of depression (Dryman and Eaton, 1991; Hagerty *et al*, 1997), and recovered patients sometimes continue to show memory impairment (Roiser *et al*, 2003). However, at the symptom level, these deficits have been found to be relatively unstable over the course of depressive illness (Oquendo *et al*, 2004). Some authors suggest that working memory deficits in depression are due to persistent deficits in selective attention (state-independence) assessed by the Stroop paradigm that have been related to persistent abnormalities in the prefrontal cortex (Trichard *et al*, 1995; Blumberg *et al*, 2003). In contrast, deficits in long-term storage and retrieval of declarative memory have been associated with number of depressive episodes, stress, hypercortisolemia, and hippocampal volume reduction found in major depression (MacQueen *et al*, 2002, 2003), thus suggesting that these memory symptoms are rather a consequence than an etiologic factor of depression.

There is a wealth of knowledge on the genetics of working memory in mice providing converging evidence that glutamate, acetylcholine and dopamine receptors, calcium/calmodulin kinase II and protein kinase C gene families play roles in short-term information processing (Luciano *et al*, 2003). In healthy humans and schizophrenic patients, a functional polymorphism of the COMT gene appeared to explain some of the individual variance in working memory performance (Egan *et al*, 2001; Malhotra *et al*, 2002). However, there is a lack of information on the heritability, familial association, and cosegregation of learning and memory symptoms related to MDD.

Neurovegetative Signs

Although appetite and weight change are not specific symptoms of major depression (Nelson and Charney, 1981), there is some evidence that the direction of appetite and weight change may be useful as a marker of depressive subtypes. The direction and extent of weight change was found to be consistent across episodes of MDD (Stunkard *et al*, 1990) suggesting some state-independence, although a recent study did not show any correlation between Hamilton depression scale appetite measures across episodes (Oquendo *et al*, 2004). Evidence for heritability and familial association comes from studies in identical twins showing that melancholic and atypical depressive subtypes independently aggregate in families and are at least in part genetically determined, and that these subtypes are most

clearly distinguished by a different pattern of appetite and weight change (Kendler *et al*, 1996). Finally, appetite-associated endophenotypes for depression seem to be biologically plausible given that brain monoamines and peptides that play major roles in major depression, such as serotonin, norepinephrine, dopamine, neuropeptide Y, and corticotropin-releasing hormone, also play important roles in the regulation of food intake and body weight (Gillard *et al*, 1993; Meguid *et al*, 2000; Wang *et al*, 2001).

More than 90% of depressed patients complain about impairments of sleep quality, and insomnia was found to be a prominent risk factor for subsequent development of depression. However, sleep disturbances including morning awakenings are not specific for major depression and co-occur with a wide range of physical and psychological symptoms. In addition, studies comparing subtypes of depression such as unipolar, melancholic, and bipolar depression failed to find consistent differences in nocturnal sleep patterns (Riemann *et al*, 2001). However, due to the availability of sophisticated examination methods such as polysomnography, sleep pathophysiology may be well suited to discover endophenotypes for major depression (see below).

Diurnal Variation

Some symptoms of MDD may show diurnal variations (mood, psychomotor activity, accessibility of memories of positive and negative experiences), and a subgroup of patients with MDD may have a circadian rhythm disorder (Bunney and Bunney, 2000). In healthy young subjects, moderate changes in the timing of the sleep-wake cycle had specific effects on subsequent mood (Boivin *et al*, 1997). The association between phase advance of the sleep-wake cycle and phase advances in nocturnal cortisol secretion, and the effect of antidepressants on circadian rhythms of behavior, physiology, and endocrinology contribute to the biological plausibility of this putative endophenotype (Duncan, 1996; Bunney and Bunney, 2000). Because manipulations of the circadian rhythms (light therapy, sleep deprivation, phase advance treatment) can have antidepressant efficacy, circadian abnormalities have been hypothesized to be etiologically associated with major depression.

There is strong evidence for a genetic control of the human circadian clock (Linkowski *et al*, 1993), and a familial variant of human sleep behavior has been attributed to a mutation in a human clock gene (Toh *et al*, 2001). The influence of a functional polymorphism within the angiotensin I-converting enzyme (ACE) gene on partial sleep deprivation in patients with MDD is probably mediated by dopaminergic neurotransmission (Baghai *et al*, 2003). A functional polymorphism within the promoter of the serotonin transporter gene has been related to sleep deprivation's efficacy and suggests an involvement of the central serotonergic system in the regulation of circadian rhythms (Benedetti *et al*, 1999).

Further epidemiological research is needed to determine state-independence, heritability, and familial aggregation of circadian abnormalities associated with MDD.

Impaired Executive Cognitive Function (Response Speed)

Impairments of executive cognitive function in depressed subjects refer to abnormalities in cognitive behaviors that control and integrate neural activities including selecting strategies, planning, and monitoring performance. These impairments are not specific for MDD and usually recover to normal levels in remission. However, response speed has been found to be unrelated to concurrent depressive symptoms and to remain impaired in fully recovered depressed patients off medication (Roiser *et al*, 2003) (state-independence). Specifically, inspection time, a measure of speed of information processing that does not require a speeded motor response, has been found to be slower in subjects with unipolar major depression than in age-, sex-, and IQ-matched controls independent of current mood (Tsourtos *et al*, 2002). The cholinergic basis of inspection time (Nathan and Stough, 2001) paralleling the hypothesis of a cholinergic dysfunction in major depression (Riemann *et al*, 1994) lends biological plausibility to this putative endophenotype. Twin studies demonstrated heritability for inspection time, sharing a substantial genetic relationship with performance IQ (Luciano *et al*, 2003).

Psychomotor Change (Retardation, Agitation)

Psychomotor retardation and agitation are included in the DSM-IV diagnostic criteria of major depression and have been shown to reliably differentiate depressed patients from psychiatric and normal comparison groups (Sobin and Sackeim, 1997). Moreover, psychomotor disturbance has been proposed as a marker of an underlying neuropathological process specific for the melancholic depressive subtype (Parker, 2000). The biological and clinical plausibility for this putative endophenotype includes associations between psychomotor disturbances and hypothalamic-pituitary-adrenocortical (HPA) axis dysfunction in depressed subjects (Mitchell *et al*, 1996), and Parkinsonian movement deficits in melancholic patients (Rogers *et al*, 2000), consistent with a potential dopaminergic dysfunction related to depression (Nestler *et al*, 2002). Unfortunately, information on state-independence, heritability, familial association, and cosegregation of specific psychomotor disturbances are lacking.

Increased Stress Sensitivity (Gender Specific)

The heritability of major depression being estimated to range between 31 and 42% emphasizes the relative importance of environmental factors (Sullivan *et al*, 2000). Given the lack of specificity between stressors and psychopathological outcomes (McMahon *et al*, 2003), one may hypothesize that gene-stressor interactions account for outcome specificity. Therefore, psychopathological constructs reflecting gene-environment interactions might be among the most specific and most useful endophenotypes for major depression. Caspi *et al* (2003) have shown in a representative prospective study that 5-HTT genotypes moderate the influence of stressful life events on major depression, thus successfully using a gene-environment interaction as phenotype.

Gender differences have appeared as a specific characteristic of stress sensitivity in humans. Men and women are, in general, equally sensitive to the depressogenic effects of stressful life events, but their responses vary depending upon the nature of the event itself. Men are more likely to have depressive episodes following divorce, separation, and work difficulties, whereas women are more sensitive to events in their proximal social network, such as difficulty getting along with an individual, serious illness, or death (Kendler *et al*, 2001).

Diathesis-stress theories of depression predict that genes influence individuals' sensitivity to stressful events (Costello *et al*, 2002), consistent with a potentially important role of gene-by-environment interactions played in the etiology of depressive psychopathology. A diagnostic construct consistent with these theories is the personality concept 'neuroticism' defined as general vulnerability to anxiety and depressive symptoms under stress. Neuroticism was found to be reasonably invariant during adulthood (Costa and McCrae, 1988), although depressive state features may have a considerable impact on neuroticism scores (Griens *et al*, 2002). There is consistent evidence of gender-specific heritability and familial association and cosegregation for neuroticism as putative endophenotype for MDD: neuroticism has been associated with gender-specific genetic factors, and a high degree of overlap between genes influencing neuroticism and major depression within the genders has been demonstrated (Fanous *et al*, 2002); the reported heritability of neuroticism is equal or greater than heritability estimates for MDD (Jang *et al*, 1996); and neuroticism has been successfully used as phenotype for genetic studies (Sen *et al*, 2003).

The biological plausibility of stress-related endophenotypes for MDD can be derived from biological correlates of increased stress sensitivity including an excessive activation of the HPA axis that is frequently found in patients with MDD (see below). Sex differences in the response of the HPA axis to stress appeared to be important: overall, women showed a greater stress responsiveness than men, consistent with the greater incidence of major depression in women (Young, 1998); moreover, men showed greater cortisol responses to achievement challenges, whereas women showed greater cortisol responses to social rejection challenges (Stroud *et al*, 2002).

Although the concept of gene-environment interactions may be most closely related to the concept of major depression, psychometric difficulties, and the complexity and temporal structure of its underlying molecular mechanism complicate its practical use. It is particularly noteworthy that recent studies have demonstrated non-genomic transmission across generations of not only maternal behavior but also stress responses (Francis and Meaney, 1999). This has clear parallels in clinical population. For example, environmental events (for example, early childhood stressors) correlate with the development of psychiatric disorders in adults (Heim and Nemeroff, 2001). Indeed, as witnessed by multiple studies of discordant monozygotic twins where one has the disorder and the other does not, epigenetic mechanisms must be operative to control behavior in genetically identical populations

(Petronis *et al*, 2003). A critical question involves the mechanism by which early life events regulate long-term changes in behavior and sustained differences in gene expression.

In most areas of the brain, neurons are not replaced; thus, permanent and semipermanent modifications that occur in early life, which alter gene transcription, could have downstream effects that may be temporally distant from the initial event. These nongenetic (epigenetic) mechanisms of gene regulation likely play a role in the formation of cellular memory and the modulation of neural circuitry in a manner that alters lifetime cellular and behavioral responses in an organism. The true extent of the dynamic mechanisms responsible is unknown and is an active area of research. However, it is known to involve the interplay of transcription factors interacting with covalent DNA modifications, such as cytosine methylation, and the accessibility of DNA that is regulated by histone acetylation (Petronis, 2001; Geiman and Robertson, 2002). These mechanisms are likely involved in modulating how previous experience may regulate subsequent behavioral responses.

BIOLOGICAL ENDOPHENOTYPES

The number of genes involved in a phenotype is thought to be associated with both the complexity of the phenotype and the difficulty of the genetic analysis (Gottesman and Gould, 2003). Assuming a large number of genes involved in the pathogenesis of major depression, and assuming that disease progression and medication may alter the clinical phenotype, defining endophenotypes for depression by narrow psychopathological definitions such as circadian abnormalities and direction of vegetative symptoms, by personality constructs such as neuroticism and reward dependence, or by neuropsychological tests including inspection time and the Stroop paradigm may still require relatively large samples to detect the effect of a single genetic polymorphism. One emerging strategy that may circumvent some of these difficulties is the use of quantitative biological markers, which are associated with disease and may be closer to single gene effects than clinical phenotypes (Almasy and Blangero, 2001). The ideal biological endophenotype would be a biological marker that is easy to assess and that reflect the action of a single gene.

The use of biological endophenotypes has been successful in locating genes for nonpsychiatric disorders. For example, some of the multiple genes involved in cardiac arrhythmias were identified using an ECG endophenotype. Initially, a prolonged QT interval on the electrocardiogram was found in some patients with familial cardiac arrhythmia. Subsequent discovery of an increased prevalence of a prolonged QT interval among healthy relatives of arrhythmic patients than among unrelated controls suggested heritability of this ECG trait in these families. Finally, using QT interval elongation as endophenotype allowed for successful genetic linkage studies (Keating *et al*, 1991; Keating and Sangiunetti, 2001).

In the following, we will present biological correlates for major depression that meet some of the endophenotype criteria.

REM Sleep

Reduced REM latency, higher REM density, and more REM sleep in adolescents turned out to be specific and predictive for unipolar major depression, whereas none of these signs were found in adolescents who later switched to bipolar disorder and in those who remained free from psychopathology at follow-up (Rao *et al*, 2002). Early changes of REM sleep parameters, especially REM latency and percentage amount of REM during the sleep period, have been found in patients with acute and remitted major depression (Giles *et al*, 1993) (state-independence). The first-degree relatives of unipolar depressed patients showed a significantly higher REM density than controls, suggesting that genetic factors contribute to these REM sleep alterations (Giles *et al*, 1998; Modell *et al*, 2003). Nearly all effective antidepressant medications have shown a pronounced inhibition of REM sleep (Murck *et al*, 2003), and the serotonergic and cholinergic neurotransmitter systems have been implicated in both REM sleep regulation and MDD, thus providing evidence for the clinical and biological plausibility of this putative endophenotype.

Several candidate genes may be considered for REM sleep-related endophenotypes. The CREB gene has been implicated in the regulation of REM sleep (Graves *et al*, 2003), memory consolidation, and major depression (Zubenko *et al*, 2002). The effect of the muscarinic cholinergic 2 receptor gene that has been implicated in MDD in women (Comings *et al*, 2002) may conceivably be mediated by REM sleep disturbances. Finally, genes associated with neurological disorders affecting REM sleep characteristics such as narcolepsy may contribute to REM sleep disturbances in MDD: the HLA class II allele DQB1*0602, genes for the hypocretin receptors, and polymorphisms in genes of the COMT and the tumor necrosis factor system (Taheri and Mignot, 2002).

Abnormalities in Brain Structure and Function

The identification of pathologic lesions in specific regions of the central nervous system has importantly contributed to the rapid progress in the understanding and treatment of neurological disorders including Parkinson's and Alzheimer's disease. Neuropathological findings are extremely useful to define nosological subtypes reflecting different underlying disease states that may be related to genetic vulnerability factors. For example, clinical features alone can only be used to diagnose parkinsonism; postmortem examination is needed for the definite diagnosis of the underlying disease including classic Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, and frontotemporal dementia (Giasson and Lee, 2003).

Although there is no consensus in the field about the site of pathology in major depression, functional, structural, and molecular brain mapping in major depression have the potential to bridge the gap between clinical depressive features and genes. Since approximately 70% of all genes are expressed in the brain, many functional gene polymorphisms can potentially influence how the brain processes information. Since functional imaging has the capacity to assay within individuals information processing in discreet brain circuits, it has the potential to provide

endophenotypes for MDD (Hariri and Weinberger, 2003). For example, significant differences have been reported between s/s and l/l genotypes of the 5-HTT promoter gene regarding the amygdala response to fearful faces measured by functional MRI in the absence of behavioral differences (Hariri et al, 2002). Furthermore, growing evidence suggests that major depression travels with brain structural changes mediated by hypercortisolemia, glutamate neurotoxicity, stress-induced reduction in neurotrophic factors, and stress-induced reduction in neurogenesis (Sheline, 2003). Therefore, advanced imaging technology might describe subtle changes in brain structures that are associated with specific pathophysiological processes and genes. Finally, *in vivo* molecular imaging opens up the potential to connect findings in genetic neuroscience obtained from animal experiments and postmortem human studies to clinical characteristics of depressed subjects by defining endophenotypes at the molecular level (eg receptors, transporters, and enzymes).

Functional imaging. A series of studies have consistently found that the resting CBF and glucose metabolism in the amygdala in subjects with familial pure depressive disease and the melancholic depressive subtype is increased (Drevets, 2000). Elevation of resting amygdala CBF and metabolism may prove specific to primary mood disorders, insofar as this abnormality has not been reported in obsessive-compulsive disorder, panic disorder, phobic disorders, schizophrenia, or other neuropsychiatric conditions (Charney and Drevets, 2002). Although the magnitude of the amygdala activity in depression is partly modulated by illness severity, preliminary data suggest that left amygdala activity is abnormally elevated in remitted depressed patients off medication with a family history for affective disorders (Drevets et al, 1992). Moreover, one study showed that increased amygdala activity predicted return of depressive symptoms in remitted MDD subjects on medication following tryptophan depletion (TD; Bremner et al, 1997). The associations between elevated amygdala activity and vegetative depressive symptoms, plasma cortisol (Drevets et al, 1992), and REM sleep (Nofzinger et al, 1999) underline the plausibility of this biological marker for MDD. However, whether elevated amygdala activity meets the endophenotype criteria heritability, familial association, and cosegregation has not yet been determined.

In the subgenual prefrontal cortex, decreased CBF and metabolism have been consistently implicated by numerous studies in MDD (Drevets, 2000; Kegeles et al, 2003). Dysfunction of this brain region has been associated with blunted hedonic response and exaggerated stress responsiveness (Pizzagalli et al, 2004), and functional alterations in this region have been observed following serotonergic challenge (Kegeles et al, 2003), induced sadness (Mayberg et al, 1999), and treatment with a serotonin reuptake inhibitor (Buchsbaum et al, 1997). In remitted depressed patients, mood provocation produced a CBF decrease in brain regions connected with the subgenual prefrontal cortex (Liotti et al, 2002). Although information about this finding in healthy subjects at risk for MDD is lacking, it represents another promising imaging endophenotype.

Structural imaging. Volume reductions in the ACC located ventrally ('subgenual') and anterior ('pregenual') to the genu of the corpus callosum have been implicated by numerous studies of mood disorders (Drevets, 2001). Specifically, a volume reduction in the left subgenual ACC has been associated with familial affective disorders by MRI-morphometric measures (Drevets et al, 1997; Hirayasu et al, 1999) and by postmortem neuropathological studies, which have shown glial reduction in the corresponding gray matter (Öngür et al, 1998). This reduction in volume exists early in the illness (state-independence), but appears to become more pronounced following illness onset, based upon preliminary evidence in twins discordant for MDD (Botteron et al, 1999). Humans with lesions that include the ventral ACC show abnormal autonomic responses to emotional stimuli, an inability to experience emotion related to concepts, and inability to use information regarding the probability of aversive social consequences vs reward in guiding social behavior (Damasio et al, 1990). In rats, left-sided lesions of the ACC increase sympathetic arousal and corticosterone responses to restraint stress (Sullivan and Gratton, 1999). The mechanisms and genes underlying volume loss in the ACC have not yet been determined. Preclinical studies on the role and genetics of neurotrophic factors and the signaling cascade neurotrophic factor/MAP kinase/bcl-2 involved in the fine balance maintained between the levels and activities of cell survival and cell death factors (Manji et al, 2003b) may inform clinical studies associating ACC volume loss to genes.

Reductions in hippocampal volumes associated with MDD have been consistently reported. However, this structural abnormality is not a specific sign of MDD (ie it has been found in patients with PTSD, schizophrenia, and epilepsy) and only occurs in a subgroup of MDD patients (Sheline and Mintun, 2002). In some studies, the volume loss appears to have functional significance with an association between acute depression and abnormalities of declarative memory (Burt et al, 1995) as well as associations between depression in remission and lower scores on tests of verbal memory (Sheline et al, 1999). The pathogenesis of hippocampal volume reduction seems to overlap with depressive pathophysiology, given its association with early-life stress, stress hormones, and duration of depressive illness (McEwen, 1999; Brunson et al, 2001; MacQueen et al, 2003). A study in monkeys suggests that hippocampal volume also reflects an inherited characteristic of the brain associated with increased cortisol response to stress (Lyons et al, 2001). Preliminary results on the genetics of the hippocampal function suggest that the BDNF val66met polymorphism may influence the development of memory deficits associated with psychopathology (Egan et al, 2003), and targeted disruption of mineralocorticoid receptor (MR) genes resulted in impaired hippocampal neurogenesis (Gass et al, 2000).

The literature is in disagreement regarding amygdala volumes in mood disorders, possibly due to technical limitations such as low reliability and validity of amygdala volume measures. However, a recent study using high-resolution 3T MRI has consistently found decreased amygdala volume in symptomatic and remitted mood disorders (Drevets et al, 2004). Amygdala volumes have

been negatively associated with amygdala activity, suggesting that amygdala hyperactivity could be a factor in amygdala volume reductions in MDD (Siegle *et al*, 2003). This association and the high clinical plausibility of neuropathological abnormalities of the amygdala as endophenotype for MDD encourages further investigation on the consistency, specificity, familiarity, and heritability of these findings.

Receptor pharmacology. Decreased 5-HT_{1A} receptor binding potential has been consistently found in multiple brain areas of patients with MDD (Drevets *et al*, 1999, 2000). This abnormality is not highly specific for MDD and has been found in patients with panic disorder (Neumeister *et al*, 2004) and temporal lobe epilepsy (Toczek *et al*, 2003), and may explain the considerable comorbidity among these conditions. The lack of effect of selective serotonin reuptake inhibitor treatment and hydrocortisone challenge on 5-HT_{1A} receptors in recovered patients with MDD suggests state-independence of this abnormality (Bhagwagar *et al*, 2003, 2004). Unfortunately, no information is available on the heritability, familial association, and cosegregation of 5-HT_{1A} receptor binding potential. However, a recent report suggests that a polymorphism associated with 5-HT_{1A} receptor transcription is more common in MDD than in controls (Lemondé *et al*, 2003).

There is growing evidence from animal studies for the biological plausibility of this putative endophenotype for stress-related disorders. Mice with mutation in the 5-HT_{1A} receptor gene have been consistently found to display increased stress-like behaviors and to express decreased activity toward a novel object (Bakshi and Kalin, 2002). However, the use of a tissue-specific, conditional rescue strategy revealed that the expression of the 5-HT_{1A} receptor in the hippocampus and cortex (but not in the raphe nuclei) during the early postnatal period (but not in the adult) is sufficient to rescue the normal behavioral phenotype of the knockout mice (Gross *et al*, 2002), suggesting that stimulation of the 5-HT_{1A} receptor during the postnatal period leads to long-lasting changes in the brain structure that are necessary for normal affective behavior throughout life.

Serotonin, Dopamine, and Norepinephrine

Alterations in noradrenergic and serotonergic function in the brain have been implicated in the pathophysiology of depression and the mechanism of action of antidepressant drugs (Charney, 1998), although dysfunctions within the monoaminergic neurotransmitter systems are not likely to play primary roles in the pathophysiology of depression but rather represent the downstream effects of other, more primary abnormalities (Manji *et al*, 2003b). Because depletion of catecholamines or serotonin induces significant depressive symptoms in remitted depressed subjects, depressive reactions in response to lowering of brain monoamine neurotransmitters has been proposed as an endophenotypic vulnerability marker for major depression (Berman *et al*, 1999).

Serotonin. Major depression has been associated with abnormally reduced function of central serotonergic systems by abundant evidence from biochemical, challenge,

imaging, and postmortem studies (Coppen *et al*, 1973; Charney *et al*, 1981; Lopez *et al*, 1998; Drevets *et al*, 1999). TD is an instructive paradigm for investigating the relationship between serotonergic function and depression. This paradigm involves the mood response to serotonin depletion, achieved by oral loading with all essential amino acids except the 5-HT precursor, tryptophan. The transient reduction in plasma tryptophan concentrations, cerebral serotonin synthesis, and central 5-HT concentrations resulting from this dietary manipulation is associated with redevelopment of depressive symptoms in remitted depressed patients who are either off medication (Delgado *et al*, 1994) or medicated with selective serotonin reuptake inhibitors (Delgado *et al*, 1999).

TD-induced depressive symptoms show a relatively high specificity for major depression (Bell *et al*, 2001). The presence of this diagnostic sign in remitted patients suggests state-independence. TD-induced depressive symptoms seem to be heritable: in remitted depressed patients, the long allele of the serotonin transporter gene promoter polymorphism predicted depressive response to TD (Moreno *et al*, 2002), while, in healthy women, the s-allele of this functional polymorphism and a positive family history of depression represented additive risk factors for TD-induced depressive symptoms (Neumeister *et al*, 2002). There is also evidence for familial association and cosegregation: never-depressed subjects with a positive family history of depression have also been shown to experience mood symptoms following TD that were smaller than in remitted depressed patients but different from subjects without familial risk who showed no effect following TD (Benkelfat *et al*, 1994).

In vulnerable individuals, TD induces mood-congruent memory bias (Klaassen *et al*, 2002), alters reward-related behaviors (Rogers *et al*, 2003), impairs memory consolidation (Riedel *et al*, 2002), slows responses to positive stimuli, and disrupts inhibitory affective processing (Murphy *et al*, 2002). These TD-induced changes are comparable with those occurring in major depressive episodes, thus implicating clinical plausibility. Acute severe serotonin depletion leads to biological changes associated with MDD, including enhanced norepinephrine transporter mRNA levels and reduced serotonin transporter mRNA levels (Koed and Linnet, 2000), increased number of MR binding sites (Semont *et al*, 1999), and altered BDNF gene expression in the dentate gyrus (Zetterstrom *et al*, 1999), thus implicating biological plausibility.

Norepinephrine/dopamine. MDD has been associated with noradrenergic and dopaminergic dysfunction. Findings implicating catecholaminergic dysfunction in the pathophysiology of depression include studies about neurotransmitter synthesis and neurotransmitter storage, showing that reduction of catecholamine stores results in an exacerbation of depressive symptoms (Mendels and Frazer, 1974).

An instructive paradigm for investigating the relationship between catecholaminergic function and depression has involved the mood response to catecholamine depletion (CD), achieved by the administration of the tyrosine hydroxylase inhibitor α -methylparatyrosine (AMPT). Mood responses to CD in healthy subjects are usually not

significant (Salomon *et al*, 1997). Among untreated, symptomatic depressed patients prior to initiation of an antidepressant treatment CD failed to exacerbate depression (Miller *et al*, 1996b). This finding may be due to the brain catecholamine function being already maximally dysfunctional in symptomatic depressed patients (ceiling effect) (Lambert *et al*, 2000). Among treated depressed patients, CD reversed the antidepressant effects of antidepressants, in particular of catecholamine reuptake inhibitors (Miller *et al*, 1996a) and light therapy (Neumeister *et al*, 1998).

Because CD has not been systematically used in high-risk subjects and across different neuropsychiatric disorders, there is a lack of information on the specificity, familial association, and cosegregation of CD-induced symptoms for MDD. The presence of CD-induced depressive symptoms in unmedicated remitted patients with MDD suggests state-independence of this biological marker (Berman *et al*, 1999).

The depressive symptoms evoked by CD were often similar to those the patients had experienced during their depressive episode, suggesting clinical plausibility. There is also evidence for biological plausibility: the CD-induced return of depressive symptoms has been associated with decreased brain metabolism in orbitofrontal and dorsolateral prefrontal cortex; increased resting metabolism in prefrontal cortex and limbic areas have been found to increase vulnerability to CD-induced depressive exacerbation (Bremner *et al*, 2003).

HPA Axis and CRH

Altered HPA axis physiology and dysfunctions of the extrahypothalamic CRH system have been consistently found in humans with major depression. There is accumulating evidence that altered stress hormone secretions in depression are more than epiphenomenal, and that antidepressants may act through normalization of these changes. Given the potentially causal role of HPA axis and CRH system dysfunctions in depressive pathophysiology, and the involvement of cortisol and CRH in a variety of biological and behavioral components of major depression, indicators of these dysfunctions are likely to be useful as endophenotypes for major depression.

Cortisol. Negative feedback regulation of the HPA axis occurs through a dual-receptor system of MRs and glucocorticoid receptors (GRs). Decreased limbic GR receptor function (Modell *et al*, 1997; Mizoguchi *et al*, 2003) and increased functional activity of the MR system (Young *et al*, 2003) suggest an imbalance in the MR/GR ratio in stress-related conditions such as MDD. The possible antidepressant properties of GR antagonists also appear compatible with the corticoid receptor hypothesis (Belanoff *et al*, 2002).

The neuroendocrine function test that measures dysfunctional cortisol responses in major depression most sensitively combines the dexamethasone suppression test and the CRH stimulation test (dex/CRH test) (Heuser *et al*, 1994). The specificity of this test is high to discriminate between healthy subjects and psychiatric patients including patients with panic disorder, mania, and schizophrenia, and its sensitivity for MDD is above 80%, depending on age and

gender; however, its specificity for MDD with regard to other psychiatric disorders is low (Heuser *et al*, 1994). Abnormal cortisol responses in MDD patients, MDD high-risk probands, and healthy controls were found to be surprisingly constant over time (Modell *et al*, 1998), and independent of the actual depressive state (Zobel *et al*, 1999), suggesting that this marker is state-independent. Findings in healthy subjects at high familial risk for affective disorders (Holsboer *et al*, 1995) suggest familial association and cosegregation.

Interactions between cortisol and its receptors and neurotransmitters, neuropeptides, and brain circuits associated with depressive symptomatology suggest biological plausibility of the dex/CRH test as endophenotype for MDD. Specifically, the MR system seems to control the sensitivity of the CRH-1 system (de Kloet, 2003), which is thought to be altered in MDD, and to be the primary mediator of 5-HT_{1A} downregulation after chronic stress (Kuroda *et al*, 1994), whereas the GR seems to be the primary receptor involved in stress-related 5-HT_{2A} receptor upregulation (Karten *et al*, 1999).

Depression-like alterations of functions of the prefrontal cortex such as inhibitory control, attention regulation, and planning following cortisol administration, and the bidirectional associations between amygdala activity and cortisol levels (Gold *et al*, 2002) suggest clinical plausibility of cortisol-related endophenotypes for MDD. Furthermore, elevated cortisol may mediate between major depression and its medical long-term consequences such as coronary heart disease, type II diabetes, and osteoporosis.

Finally, there is some preliminary evidence for the heritability of GR/MR system functions. Some humans manifest a relative glucocorticoid resistance caused by GR gene mutations (Koper *et al*, 1997). In mice, conditional GR overexpression has been suggested as a model for increased anxiety-related behavior not secondary to altered levels of stress hormones (Muller *et al*, 2002). Targeted disruption of MR genes resulted in impaired hippocampal neurogenesis (Gass *et al*, 2000). Genetic factors most probably act in concert with environmental factors: an increase in hippocampal MR levels has been shown after exposure to psychological stress (Gesing *et al*, 2001).

Corticotropine-releasing hormone. The evidence for the specificity, and clinical and biological plausibility of endophenotypes related to dysfunctions of the hypothalamic and extrahypothalamic CRH system for MDD is abundant: Levels of CRH in the CSF are elevated in some depressed subjects (Nemeroff *et al*, 1984), while the pituitary response to CRH appears appropriate given the high cortisol levels (Gold *et al*, 1986); the number of CRH-secreting neurons in limbic brain regions is increased (Raadsheer *et al*, 1994); the number of CRH binding sites in the frontal cortex is reduced, possibly as a compensatory response to increased CRH concentrations (Nemeroff *et al*, 1988); CRH produces a number of physiological and behavioral alterations that resemble the symptoms of major depression including decreased appetite, disrupted sleep, decreased libido, and psychomotor alterations (Nemeroff, 1996); and anxiety and depression scores have been reduced following CRH-1 receptor blockade (Zobel *et al*, 2000).

One of the strongest models of environmental regulation of the development of responses to stress is the postnatal handling research. The central CRH system is seen as the critical target for these environmental effects (Francis and Meaney, 1999). Postnatal maternal separation increases CRH gene expression in the paraventricular nucleus and alters systems involved in the regulation of the CRH system (eg the noradrenergic system); these effects may become permanent. Additionally to these environmental regulations, genetic factors have to be taken into account: reports on mouse mutants where CRH receptors were genetically inactivated suggested that CRH-R1 mediates anxiety-like behavior (Timpl *et al*, 1998); the CRH binding protein gene has been found to be involved in the vulnerability for MDD (Claes *et al*, 2003).

Although the CRH system is among the most promising to provide clinically and biologically plausible endophenotypes for MDD, few studies have investigated the temporal stability, heritability, familial association, and cosegregation of the CRH system in relation to major depression. One reason for the lack of such studies is the lack of PET ligands for CRH receptors, which can permit noninvasive assessments of CRH system dysfunction *in vivo* in humans.

Intracellular Signaling Molecules (a Perspective)

Neurotrophic factors. The prominent cognitive deficits and brain volumetric changes in depression give additional weight to the contention that severe mood disorders arise, at least in part, from impairments of cellular plasticity and resilience (Manji *et al*, 2001; Manji and Duman, 2001). Endogenous neurotrophic factors such as BDNF are necessary for growth, survival, and functioning of neurons. They increase cell survival by providing necessary trophic support for growth, and also by exerting inhibitory effects on cell death cascades. There is emerging evidence, primarily from postmortem studies, that supports a role for abnormalities in neurotrophic signaling pathways in depression. Decreased levels of CREB, BDNF, and the TrkB receptor have been reported in suicide victims (Odagaki *et al*, 2001; Dwivedi *et al*, 2003).

As discussed already, genetic abnormalities in CREB and BDNF may also occur in depression. Substantial evidence indicates that CREB is a core component of the molecular switch that converts short- to long-term memory. A growing body of evidence suggests that antidepressants may regulate neurotrophic signaling cascades. Antidepressant treatment in rats increases CREB phosphorylation and CREB-mediated gene expression in mice limbic brain regions. More evidence that relates upregulation of these pathways and antidepressant utilization comes from antidepressant-like performance in behavioral models. Thus, it was observed that CREB overexpression in the dentate gyrus or BDNF injection results in an antidepressant-like effect in the learned-helplessness paradigm and the forced swim test model of antidepressant efficacy in rats (Chen *et al*, 2001; Shirayama *et al*, 2002). Together, the data suggest that alterations of neurotrophic signaling cascades may underlie the pathophysiology and treatment of depression. Because of the potential genetic underpinnings of abnormalities of these neurotrophic pathways, they may provide biological endophenotypes for major depression.

Ubiquitous signaling cascades. It is now clear that genetic abnormalities in signaling components are often fully compatible with life, and in many instances, despite the often-ubiquitous expression of the signaling protein, these abnormalities are associated with circumscribed clinical manifestations (Manji *et al*, 2003a). These overt, yet relatively circumscribed, clinical manifestations are believed to ultimately arise from vastly different transcriptomes (all of the transcripts present at a particular time) in different tissues because of tissue-specific expression, haploinsufficiency, genetic imprinting, alternate splicing, varying stoichiometries of the relevant signaling partners in different tissues, and differences in the ability of diverse cell types to compensate for the abnormality (either autocrine or paracrine). Moreover, there is preliminary evidence that abnormalities in shared signaling cascades may even play a role in the growing appreciation that 'comorbidities' of major depression including cardiac disease, migraines, atopic disease, type II diabetes, and anxiety disorders appear to be the rule rather than the exception. There is no question that some medical illnesses (eg osteoporosis) represent the secondary sequelae of the biochemical changes (eg hypercortisolemia, sympathetic hyperarousal) of depression; however, some comorbidities may arise because the signaling cascade (eg cAMP cascade) also plays a role in the pathophysiology of these other disorders (eg vascular reactivity in migraine). In support of such a contention, Bondy *et al* (2002) investigated the ACE ID and the G-protein beta3-subunit (Gbeta3) C825T polymorphism in 201 patients with major depression and 161 ethnically and age-matched controls. Both gene variants have earlier been implicated in cardiovascular disease or affective disorders, making them good candidates for a combined analysis. Analyzing the data for both genes, they found that the combined actions of ACE and Gbeta3 genotypes accumulate in carriers of the ACE D allele (ID and DD) and Gbeta3 TT homozygotes, with ID/DD-TT carriers showing a more than five-fold increase in risk for major depression. These intriguing findings suggest that heritable biological makers of common medical conditions may turn out to be useful endophenotypes for major depression.

CONCLUSIONS

Table 1 shows an overview of putative psychopathological and biological endophenotypes for MDD, indicating estimates of evidence for each putative endophenotype with respect to the endophenotype criteria (Tsuang *et al*, 1993).

Anhedonia-related endophenotypes showed good evidence regarding endophenotype criteria including specificity. These endophenotypes parallel Klein's 'endogenomorphic' depressive subtype reflecting 'inhibited pleasure mechanisms' as psychopathological core feature (Klein, 1974). However, longitudinal and high-risk studies using specific anhedonia measures and tasks that estimate abnormalities of the brain reward system are necessary to further evaluate the applicability of anhedonia-related endophenotypes for major depression.

Increased stress sensitivity also met most of the endophenotype criteria. Particularly, the familial coaggregation with MDD makes this phenotypic construct qualified

Table 1 Evaluation of Putative Endophenotypes for Major Depression^a

	Specificity	State-independence	Heritability	Familial association	Cosegregation	Plausibility	Total
<i>Psychopathological endophenotypes</i>							
Depressed mood (mood bias)	+	+	0	+	0	+++	6
Anhedonia (impaired reward function)	+	+	+	++	+	+++	9
Impaired learning and memory	±	+	0	0	0	+	2
Direction of appetite change	–	±	+	+	0	++	4
Diurnal variation	++	+	+	0	0	++	6
Exec. cogn. function (response speed)	+	+	++	+	0	+	5
Psychomotor change	–	–	0	0	0	+	1
Increased stress sensitivity	–	+	++	++	+	+++	9
<i>Biological endophenotypes</i>							
REM sleep abnormalities	±	+	+	++	+	++	7
Increased amygdala activity	++	+	0	0	0	+++	6
Decreased subgenual PFC activity	+	+	0	0	0	+++	5
Left ACC volume reduction	+	++	0	++	0	++	7
Hippocampal volume reduction	–	++	+	+	0	++	6
Reduced 5-HT _{1A} receptor BP	+	++	+	0	0	+++	7
Tryptophan depletion	+++	+++	++	++	++	+++	14
Catecholamine depletion	+	+++	0	0	0	+++	7
dex/CRH test	±	++	++	++	++	+++	11
CRH dysfunction	+	+	+	0	0	+++	6

^a–, one or more studies did not support this finding (with no positive studies), or the majority of studies do not support this finding; ±, equal number of studies support this finding and do not support this finding; +, at least one study supports this finding and no studies do not support this finding, or the majority of studies support this finding; ++, two or more studies support this finding, and no studies do not support this finding; +++, three or more studies support this finding, and no studies do not support this finding; 0, data not available.

for genetic studies; however, its use is limited by low specificity for MDD and by psychometric issues: the genetics of the partly state-dependent construct ‘neuroticism’, which can easily be assessed by a questionnaire, may be as complex as the genetics of MDD, and a potentially more reliable and more valid assessment of stress sensitivity by measuring stress levels and stress symptoms over time by means of a prospective community-based study is extremely costly and time-consuming.

Among the biological endophenotypes, TD showed evidence for all of the endophenotype criteria including specificity, state-independence, and familial association, thus encouraging the use of TD to identify a potentially homogenous depressive subtype associated with serotonergic dysfunction. Unfortunately, the complexity of TD including the use of a pharmacological challenge, the clinical observation of the research subject over many hours, and the exclusion of patients with symptomatic depression reduce the usefulness of this endophenotype, particularly in epidemiological research. The dex/CRH test, being somewhat easier to apply than TD, also showed good evidence across various endophenotype criteria. The low specificity for MDD, however, represents a major limitation to the use of this biological marker as MDD endophenotype.

The large sample sizes of MDD patients needed to perform genetic association studies on subsamples stratified according to endophenotypes is a general limitation to the endophenotype approach. In addition, using PET or fMRI

endophenotypes may not be practical given the huge costs to obtain a sample size with sufficient power for genetic studies. The systematic evaluation of combinations of related psychopathological and biological endophenotypes (eg attentional bias toward negative stimuli combined with functional/structural abnormalities in the subgenual prefrontal cortex) expands the scope of this review.

FUTURE DIRECTIONS

Given the relative scarcity of well-designed twin, family, and prospective studies evaluating putative psychopathological and biological endophenotypes (see Table 1), future research has the potential to improve considerably the phenotypic definition of MDD. Progress in developing economical and easy-to-apply neurobiological markers may considerably facilitate the discovery of biological endophenotypes. Moreover, in the long term, the development of a new diagnostic system that includes psychopathological and biological endophenotypes will be necessary to improve the power of genetic studies by systematically defining relatively homogenous depressive subtypes.

The Framingham Heart Study, designed as a large community study with a long-term follow-up, helped to identify biological and behavioral risk factors for cardiovascular disease and to establish diagnostic criteria for nosological entities such as arterial hypertension. Likewise,

prospective longitudinal studies that collect comprehensive phenotypic data of psychiatric disturbances (eg the Zurich Cohort study; Angst *et al*, 1984; Hasler *et al*, 2004) are required. These studies are very different from the conventional phenotypic data collection (DSM-disease present or absent); it is rather a systematic effort to quantify the manifestations that compose the overall phenotype. In addition, these long-term follow-up studies are also required to identify environmental modifiers and precipitants that may alter clinical and biological phenotypes. The definition of endophenotypes in a way that takes developmental and environmental factors into account to detect vulnerability genes (Caspi *et al*, 2002, 2003) is an exciting model for future epidemiological research in depression.

Neuroimaging, postmortem, and preclinical studies are required to discover neurobiological endophenotypes bridging the gap between behavioral phenotype and genotype. Specifically, postmortem studies on gene variation conducted in families or populations may prove very useful in studying many facets of the gene or genes in question. By linking gene and gene expression (mRNA) to brain structure and function, postmortem research can be used to identify genetically relevant disease subtypes and to validate neuroimaging findings. New PET ligands that have specificity for receptors implicated in the pathophysiology of major depression, which are sensitive to dynamic neurotransmitter function, are needed to associate baseline levels of receptor occupancy as well as receptor displacement in response to pharmacological and behavioral challenges to both behavioral phenotypes and genotypes. Dramatic progress in the development of MRI-based methods will help to reliably identify subtle abnormalities of neural structures, connectivity, and function in depressed subjects and healthy subjects at familial risk that may be used as endophenotypes for genetic studies (Charney *et al*, 2002). Finally, longitudinal neurobiological studies are required to elucidate impairments of neuroplasticity and cellular resilience in major depression (Manji and Duman, 2001).

Taken together, reducing phenotypic heterogeneity is crucial for the identification of vulnerability genes for major depression, and, therefore, the development of a new classification system is badly needed. We propose to dissect the behavioral phenotype into key components, and integrate specific environmental risk factors and neurobiological endophenotypes into the new classification system. We hope and expect that advances in epidemiology, neurobiology, and genetics will result in ongoing improvements of the phenotypic definition of major depression based on etiology and pathophysiology.

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