

The Genomic Approaches to Major Depression

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Abstract: Major depression is a chronic state of depressed mood with significant genetic predisposition. Although a lot of efforts has been tried to localize or associate the genes of major depression, etiological heterogeneity and complexity of this disorder has greatly hold back the use of traditional linkage strategies and candidate gene approaches. Recent advances in high throughput genotyping and microarray techniques have proffered opportunities for comprehensive investigation on genetic alterations and expressions on a scale not previously possible. It has become feasible to find genes with small effect size in major depression using genome-wide association scanning and to identify new pathways or mechanisms of psychopharmacology by the application of functional genomics. In the era of genomic approach to the pathophysiology of major depression, the authors suggest (1) reducing the heterogeneity of major depression by subgrouping the patients according to biological traits such as responses to antidepressant treatments, (2) searching for genes with small effect size using association scanning strategy, genomic control and DNA pooling techniques in genotyping; and (3) developing animal models with genetic vulnerability to major depression by ethylnitrosourea (ENU) mutagenesis technology and efficient behavioral screening tests.

Key Words: association, genomics, linkage disequilibrium, major depression, microarray

INTRODUCTION

Major depression is defined as a chronic state (more than two weeks) with the core symptom of depressed mood or general loss of interest, and, at least four of the following secondary symptoms: (i) significant body-weight change; (ii) insomnia or hypersomnia; (iii) psychomotor agitation or retardation; (iv) fatigue or loss of energy; (v) feelings of worthlessness or excessive guilt; (vi) diminished ability to think or concentrate or indecisiveness; and (vii) recurrent thoughts of death, suicidal ideation or suicide attempt [DSM-IV, 1994]. The treatment of depression was revolutionized in the 1950s when the efficacy of tricyclic antidepressants and monoamine oxidase inhibitors was discovered. The specific neurochemical mechanisms which underpin the therapeutic action of antidepressant medication are inhibition of the serotonin or norepinephrine-reuptake transporters or inhibition of monoamine oxidase [Frazer, 1997]. The success of this class of antidepressant has led to the development of numerous second generation medications (e.g., serotonin-selective reuptake inhibitors [SSRIs] and norepinephrine-selective reuptake inhibitors). However, after a lot of scientific investigations, our knowledge of the pathophysiology of major depression is still centered on the monoamine hypothesis. Second-generation antidepressants, relying on exactly the same mechanism as the antidepressants developed 50 years ago, do not show better therapeutic efficacy.

Apart from investigations on the pathophysiology of major depression, another approach is focused on elaboration of the most fundamental etiology, attempting to identify the genes of major depression. Although a wide range of risk

factors have been reported [Parker, 1979; Tennant, 1988; Kessler, 1997], increased risk for major depression has been repeatedly observed in relatives of individuals with this dysfunction [Gershon *et al.* 1976, 1982; Tsuang *et al.* 1980; Weissman *et al.* 1982]. The results of most twin studies also support a significant genetic contribution in the pathogenesis of major depression. The heritability was estimated from 36 to 70 percent [Kendler *et al.* 1992; McGuffin *et al.* 1996; Lyons *et al.* 1998; Kendler *et al.* 1999]. Encouraged by localization of the genes of neuromuscular diseases like Huntington's disease [Gusella *et al.* 1983] and Duchene muscular dystrophy [Ray *et al.* 1985], linkage studies using DNA markers to hunt for the genes of psychiatric diseases became the cutting edge of psychiatric genetics in the 1980s and 1990s.

TRADITIONAL LINKAGE STUDIES

Linkage studies use families with more than one affected member to determine the chromosomal locations of susceptibility genes. These investigations are capable of detecting a DNA marker 10–20 million base pairs distant from the target area, requiring only a few hundred markers for systematic screening of the genome. Further, linkage study requires no knowledge of disease pathophysiology and, therefore, offers obvious attractions for study of psychiatric illnesses where pathogenesis is poorly understood. Initially, the focus was on identifying large affected families, an approach which has been useful for disorders that follow simple, single-gene modes of inheritance. A more recent study trend involves smaller nuclear families, now recognized as more useful for investigation of complex disease [Badner *et al.* 1998]. Linkage analysis of mood disorders is difficult because of unknown model of inheritance, genetic heterogeneity, and non-genetic factors in complex (nonmendelian) diseases.

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Nonetheless, linkage analysis has been successful in localizing disease-causing genes for subtypes of complex illnesses characterized by heterogeneous etiology, such as colon cancer [Leppert *et al.* 1990; Nishisho *et al.* 1991], Alzheimer's disease [George-Hyslop *et al.* 1992; Schellenberg *et al.* 1992; Van Broeckhoven *et al.* 1992], and breast cancer [Hall *et al.* 1990; Easton *et al.* 1993]. These successes suggest that the impediments associated with complex diseases are not absolute obstacles to the discovery of susceptibility genes for nonmendelian illnesses.

Egeland *et al.* [1987] initiated the pioneer linkage study of mood disorder, reporting tight linkage to chromosome 11 in the Old Order Amish multigenerational kindred 15 years ago. To date, the search for mood-disorder genes has focused primarily on bipolar disorder because of stronger evidence for a genetic basis. In the early years, those involved in this research experienced a series of highs and lows, with encouraging findings reported but attempted replication subsequently failing. For example, Baron *et al.* (1987) tested for linkage to bipolar and unipolar disorders at the Xq28 region linked disorder in five large Israeli pedigrees and reported a maximum lod score of 9.10 coinciding with the position of the colour blindness gene, but a re-analysis carried out after three of the families were re-diagnosed and re-investigated using polymorphic DNA markers at the Xq27-28 region, resulted in a lower lod score of just above 2.00 (Baron *et al.* 1993). Kelsoe *et al.* [1989] also found a few diagnostic changes markedly attenuated the evidence for linkage between bipolar disorder and 11p15 reported by Egeland *et al.* [1987]. In a careful review, Straub *et al.* [1993] concluded that the discrepancies between the original report [Baron *et al.* 1987] and the molecular approach [Baron *et al.* 1993] to replicate the linkage between X-chromosome markers and bipolar affective illness resulted from question-able methods used in the original report, including poor quality control of G6PD assays, failure to maintain an independence between diagnosis and genotype assignment, and genotypic determinations based solely on family history. The problems of replicating linkage claims in psychiatry have been thoroughly explored by Suarez *et al.* [1994]. Nevertheless, as more of the genome has been surveyed for larger sets of families in recent years, several loci in chromo-somes 18p [Maier *et al.* 1995; Stine *et al.* 1995; Freimer *et al.* 1996; MacKinnon *et al.* 1998; McMahon *et al.* 2001], 21q22 [Straub *et al.* 1994; Gurling *et al.* 1995; Smyth *et al.* 1997; Aita *et al.* 1999], and Xq24-27 [Mendlewicz *et al.* 1979, 1980; Gill *et al.* 1992; Ekholm *et al.* 2002] have yielded reproducible evidence for linkage, which is being further explored by fine mapping and multiple data sets.

LINKAGE-DISEQUILIBRIUM MAPPING

As major depression is such a complex disorder, even responsible genes may be represented in the human population by common alleles with subtle phenotypic effects. Thus, it would be difficult to localize an individual depression gene using the traditional linkage-analysis approach. Theoretical modeling suggests that a genome-wide association scan, employing every polymorphic marker in the human genome, may have greater power to detect complex

disease-causing polymorphisms than genome-wide linkage studies, even after compensating for the increased number of false positives expected from testing such a large number of markers [Risch and Merikangas, 1996]. Association studies use unrelated affected individuals and appropriate comparison individuals, with the frequency of an allele at the marker locus for controls compared with that for the studied patients. If a specific allele of a DNA marker is close to a gene of depression on the same chromosome, they will rarely be separated by recombination, even after many generations, resulting in so-called linkage disequilibrium. For example, with a recombination fraction of 0.01 (about 1 cM or 1 million base pairs' distance) the half-life of an association can be estimated as about 70 generations or 2000 years [Morton, 1998]. This could result in demonstration of a positive association for a gene allele that does not itself cause the trait, but is in linkage disequilibrium with the actual cause. Zubenko *et al.* [2002] recently adopted this strategy to scan for a depression-susceptibility gene, determining a linkage disequilibrium between the D2S2944 marker and recurrent, early-onset major depression in women.

Until recently, systematic association scanning strategy, using hundreds of thousands of DNA markers evenly separated by about 5-100 kb (in contrast to 10-20 Mb in linkage studies) [Reich *et al.* 2001], was impossible because of the lack of sufficiently detailed marker maps. With huge numbers of SNP markers along each chromosome and large-scale genotyping technologies now available (as described in the special issue of *Psychiatric Genetics* in June 2002), the association approach, using very dense anonymous markers to scan linkage disequilibrium in a specific chromosome or even the whole genome, has become feasible. Risch and Merikangas [1996] have demonstrated that association signals can be much more numerous than linkage signals, even after Bonferroni adjustment for 1000 tests. These workers have also demonstrated that genome-wide association scans with 500,000 SNP markers may be conducted with reasonable sample sizes of 100-2000 subjects. The major limitation of systematic association-scanning strategy is the cost of the genotyping. Although recent advances in biotechnology have resulted in significantly reduced prices, the cost of genotyping 10,000 markers for 1000 cases is US \$30-60,000,000, at current prices (McGinnis, 2002).

CANDIDATE-GENE ASSOCIATION STUDIES

Conceptually, molecular genetic studies can be divided into the positional and candidate-gene approaches. Using the positional approach, the chromosomal locations of susceptibility genes are determined, usually by linkage studies. By contrast, the candidate-gene approach presupposes that the researcher has sufficient understanding of disease biology, allowing recognition of the genes that may be involved in the studied disorder. While there are functional polymorphisms or VAPSE (variation affecting protein structure or expression) in the candidate gene, the rationale of the association study will be maximized [Sobell *et al.* 1992; Craddock, 1996]. Given that monoamine dysregulation is one of the most important of the depression pathophysiologies, studies designed to explore the relationship between major depression (or its response to

antidepressant treatment) and nucleic acid variations in the genes related to the transmission, regulation or metabolism of monoamines have been abundant during the past 15 years (e.g. Hong *et al.* 1999; Lin *et al.* 2001; Liu *et al.* 2001). Although many positive findings have been reported, such as the genes encoding tryptophan hydroxylase (TPH) [Bellivier *et al.* 1998; Tsai *et al.* 1999], only a few interesting preliminary findings, including those for polymorphisms within the genes encoding catechol-o-methyl transferase (COMT) [Mynett-Johnson *et al.* 1998; Rotondo *et al.* 2002], monoamine oxidase A (MAO-A) [Preising *et al.* 2000; Schulze *et al.* 2000] and the serotonin transporter (5-HTT) [Preising *et al.* 2000; Rotondo *et al.* 2002] survive replication. Candidate-gene strategies have been unsuccessful so far, probably because of incorrect selection of the genes in question, or, because basic biological knowledge about most mental disorders, which is a prerequisite for success in such investigative endeavours, is still lacking. The high rate of false-positive findings is most probably caused by: (1) repeated tests on the same loci by independent laboratories world-wide, which is difficult to correct in advance using statistical methods; and (2) population stratification, with association relying on group differences in allele frequencies rather than familial inheritance of DNA markers. Thus, this false-positive rate may be biased by variations in the genetic make-up of the population that are unrelated to the target disorder (e.g. ethnic groups may differ in gene frequency). This problem can be overcome by means of family-based design or genomic control. Family-based design utilizes DNA obtained from a proband with major depression and both biological parents. The non-transmitted parental alleles are used as a notional control, avoiding many of the population-stratification problems associated with conventional case-control study [Falk & Rubinstein, 1987]. Genomic control uses the genome itself to determine population history and to produce appropriate corrections for population-based association tests [Devlin and Roeder, 1999]. When population substructure is absent, genomic control is more powerful than transmission disequilibrium test (TDT) [Bacanu *et al.* 2000]. Economically, genomic control is more expensive than family-based designs, because of the cost of genotyping null loci. However, recruitment costs are usually much lower for the case-control study, relative to a similarly powered family-based study. Thus, on balance, genomic control implemented by a case-control study should be at least competitive with, and probably less expensive than, family-based designs.

GENOME-WIDE FUNCTIONAL APPROACH

As significant effects have been demonstrated for antidepressant treatment of major depression, defining the roles of specific genes and/or identifying the candidate molecular pathways targeted by these medications appears to be the most direct investigative route for exploration of the biological basis of depression. Given the proposition that antidepressant action most probably involves the regulation of serotonergic and noradrenergic signal-transduction pathways [Frazer, 1997], in the last few decades many researchers have focused on discovering the receptors, ion channels, and enzymes relating to monoamine regulation, transmission, and metabolism using either biochemical

isolation, traditional cloning methods, or examination of random cDNA sequences [Ruffolo *et al.* 1991; Collins *et al.* 1990; Bobker & Williams, 1990; Vallar & Meldolesi, 1989]. Although a tremendous amount of knowledge about monoamine signal transduction has been accumulated from these previous endeavours, no consensus has been reached concerning the precise molecular and cellular mechanisms of drug action. Further, these studies have usually been conducted to characterize the products of gene transcription and translation relevant to a single pathway and a few molecules. While this approach helps to elucidate the relevance of a particular gene to a particular pathway, it cannot account for the manifestation of a single gene within the complexity of the entire system.

With the emergence of functional genomics, the emphasis has shifted to consideration of the consequences of the expression of every single gene in the nervous system to the genome in its totality. Complex diseases, involving the interaction of multiple genes and even environmental factors, can be analyzed for quantitative and coordinate levels of gene expression. The contextual information and technical capacity are now available to allow this complex analysis. The recently devised DNA-microarray method for obtaining genome-wide mRNA expression data can provide a global view of changes in gene-expression patterns in response to physiological shifts or manipulation of transcriptional regulators. For example, Landgrebe *et al.* [2002] have performed gene-expression analysis for mice treated with paroxetine and mirtazapine. These workers quantified the effects of these treatments on gene expression in the mouse brain with cDNA-microarrays containing 3624 expressed sequence tags (ESTs) and found that both drugs led to downregulation of four common genes. Their findings suggest that antidepressants with different pharmacologies can share molecular targets, even though the primary pathways through which they act are different. Bosetti *et al.* [2002] have examined the gene-expression profile for the brains of rats fed lithium chloride. Of the 4132 genes represented in the microarrays, 50 were downregulated at least two fold at 42 days, without any evidence of upregulation, while 37 of these 50 genes were not downregulated on the seventh day of treatment.

In light of the recent advances, the utilization of functional genomics represents a new phase of depression research. By making use of high-throughput technology and large-scale experimental methodologies, the information provided by structural genomics combined with statistical and computational analysis of the results affords significant expansion of the scope of single-gene and protein study to include all genes or proteins simultaneously, in a systematic fashion [Hieter & Boguski, 1997].

PERSPECTIVES AND SUGGESTIONS

Although association study can detect genes with small effect size, the sample size required for demonstration of a significant association between disease and gene depends on effect size (one measure of effect size is the ratio of disease risk in high-risk homozygotes compared with low-risk homozygotes [McGinnis, 2002]), which is especially

sensitive to the heterogeneity of the sample population. For example, the effect size for a given gene will be reduced from 2.25 to 1.6 if half the patients don't carry the susceptibility variants of this gene as might be the case in genetic heterogeneity. If the effect size of a gene with a disease allele frequency of 0.15 is reduced from 2.25 to 1.6, however, the required sample size would increase from 1300 to infinity and, therefore, the risk gene could never be detected [McGinnis, 2002]. When biological markers, such as evoked potential [Chen *et al.* press], brain imaging, therapeutic and adverse responses to various antidepressants [Wu *et al.* 2001; Hong *et al.* press; Yu *et al.* press], are used, not only may homogeneity be improved, but psychiatrically uncompromised family members who carry the susceptibility genes can be identified because these markers are less likely to be affected by psychosocial factors and may display higher penetration than the depression itself. In addition, a preliminary screening using DNA pooling and quantitating techniques (Arnheim *et al.* 1985; Carmi *et al.* 1995) to compare the estimated allele frequency in the depressed patients and controls can greatly reduce the number and cost for genotyping in a systematic association scanning approach.

While rapid progress has been achieved in the field of functional genomics, the study of psychiatric disorders, such as major depression, is facing a major obstacle, access to the target organ for application of this technology. Since it is

impossible to study the biochemical and molecular processes of the human brain under experimental control, animal models of depression are of great value. Although existing animal models remain imperfect approximations of depression, they have contributed significantly to the development of major depression treatments. Current animal models of depression rely on one of two principles, the action of known antidepressants and responses to stress [Willner, 1995; Porsolt, 2000]. Some of these tests, the forced swim test (FST) [Porsolt, *et al.* 1977] and the tail suspension test (TST) [Steru *et al.* 1985] in particular, have been very effective for prediction of the antidepressant efficacy of new medications. However, one caveat is that these tests utilize normal mice, while depression probably requires a genetic vulnerability in most cases. ENU (ethylnitrosourea) is a potent mutagen for mouse spermatogonial stem cells [Russell *et al.* 1979]. ENU mutagenesis technology will induce random mutations in the mouse germ cell line, and using rapid phenotypic screening with related devices, such as automatic FST: Supermex [Muromati; Tokyo] and PsyLab FST [Hong, ROC patent], automatic TST: ITEMATIC-TST [Steru *et al.* 1987] and PsyLab TST [Hong, USA patent pending], mutant mice susceptible to behavioral despair can be cloned. Recently, Yoshikawa *et al.* [2002] have identified multiple genetic loci for propensity to this behavior in mice. Utilizing the automatic devices mentioned above, the traits of psychomotor activity were evaluated for an F2 generation

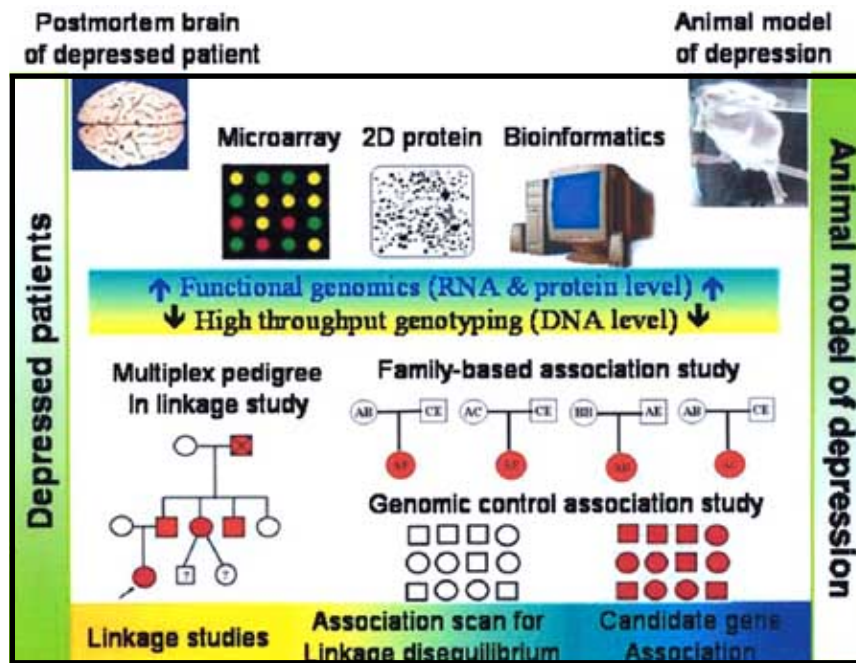


Fig. (1). Summary of the genomic approaches to major depression.

Linkage studies use multiplex pedigrees to determine the chromosomal locations of susceptibility genes; family-based designs with transmission disequilibrium test (TDT) and genomic control in case-control samples can overcome the problem of population stratification in association studies. The discovery of millions of SNPs along with the development of high-throughput genotyping technology makes systematic association scans feasible. Functional genomics, utilizing microarrays and 2-D protein electrophoresis, assay gene function by combining large-scale experimental methodologies and statistical and computational analysis of the results. The brain is the target organ for study of major depression, samples of which may be obtained from deceased patients or animals with modeled depression. Human study of major depression relies more on the use of DNA, while RNA or protein assay relies more on the development of animal models with adequate validity.

produced by intercrossing four F1 inbred strains with different psychomotor activity.

Combining genomic and proteomic tools with increasingly sophisticated animal models of depression (Fig. (1)) promises the identification of susceptibility genes for major depression and, consequently, a revolution in the understanding and conceptualization of, and clinical practice for, major depression. However, utilization of such molecular genetic techniques to identify the genes involved in such complex systems is a significant challenge. These systems are influenced by multiple genes as well as unspecified numbers of nongenetic factors, especially where a single gene is neither necessary nor sufficient. In addition, such advances must necessarily expose important ethical and psychosocial issues, including availability of the new technology and access to the genetic information. Further, the availability of this genetic information will produce a subtle shift from a simple doctor-patient relationship to a doctor-patient-family relationship. These and many other psychosocial and ethical dilemmas will require investigation in parallel with the current molecular genetic studies.

CONCLUSION

It is believed that, in the next few years, exhaustive analysis of the genome sequence of patients with depression, together with intensive study of the intricate functional genomics of the brains of modeled animals, will reveal several, perhaps many, susceptibility genes for major depression. This will greatly enhance our understanding of the pathogenesis of major depression, enabling the development of objective biological, differential diagnostics and improved treatment regimens because of enhanced targeting based on etiological fundamentals. It is likely that the impact of such achievements will lead to a revolution in clinical psychiatry of a magnitude comparable to that which followed the discovery of pharmacological modalities of treatment for mental disorders more than 50 years ago. Thus, careful consideration of the important ethical issues which will accompany these developments is important.

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