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USING HUMAN GENOMICS TO ADVANCE NEUROPSYCHOPHARMACOLOGY

L. ALISON MCINNES
NELSON B. FREIMER

Genomics, the study of genomes, includes gene mapping, sequencing, and investigation of gene functions. This field will advance neuropsychopharmacology in two complementary ways. First, it is hoped that application of genomics technologies to pedigree and population samples of patients with psychiatric disorders will allow the identification of genes contributing to the etiology and pathogenesis of these diseases and provide a rational basis for new drug development. Second, variations in the sequence of known genes whose products are the targets of current psychotropic drugs may influence the likelihood that an individual patient will have a therapeutic response to these drugs or experience a side effect. Identification and functional characterization of these sequence variants in large populations of patients of various ethnicities constitutes the discipline of pharmacogenomics. The scope of psychopharmacogenomics, however, is currently restricted by our limited knowledge of the genes that contribute to psychiatric disorders and the neural pathways altered by psychotropic agents. Fortunately, data and technologies provided by the U.S. Human Genome Project (HGP), including provision of the complete sequence of the human genome, will greatly facilitate identification of such genes (1,2). This chapter describes how technologic advances in genomics will shape the future of psychiatric genetics and psychopharmacogenomics, fields that may establish an objective basis for the restructuring of the nosology, diagnosis, and treatment of psychiatric disorders. The results of genetic studies of particular psychiatric disorders and of responses to specific drugs are considered in other parts of this book.

L. A. McInnes and Nelson B. Freimer: Department of Psychiatry and Neurogenetics Laboratory, University of California–San Francisco, San Francisco, California 94143.

IDENTIFYING GENES FOR PSYCHIATRIC DISORDERS

Rational strategies for the advancement of psychopharmacology are dependent on furthering our currently sparse knowledge of the pathophysiologic basis of psychiatric disorders. To this end, human genetic approaches offer a promising alternative to traditional biochemical and neurophysiologic investigations as twin, family, and adoption studies all support the heritability of many psychiatric syndromes. Unfortunately, attempts to first map (i.e., localize a unique region of DNA shared by patients with a particular disorder) and then identify genes predisposing to psychiatric disorders have been frustrated by the complexity of the genetic mechanisms underlying behavioral phenotypes.

A phenotype is the observable physical manifestation of genetic variation at a particular site or locus in the DNA, whereas a genotype refers to the actual DNA sequence, at the responsible genetic locus. With single gene disorders (also referred to as mendelian disorders) there is a simple, direct relationship between variation in a single gene and the phenotype that results. Thus, all patients with a given mendelian disorder, such as cystic fibrosis, will carry abnormal genotypes at a single disease locus. In contrast, the relationship between phenotype and genotype is not straightforward for complex genetic traits. In this setting, multiple different susceptibility genes and environmental factors interact in varying combinations within individuals who appear to have clinically indistinguishable phenotypes. This means that in any given sample of patients diagnosed with a particular psychiatric disorder, the number of individuals actually sharing a disease gene or genes in common might be very small such that the "effective" sample size does not provide enough power to detect the responsible genes.

Fortunately, there are strategies for finding genes contributing to complex traits that have been successfully applied to the genetic dissection of other nonpsychiatric, genetically

heterogeneous disorders. One approach is to try to reduce genetic heterogeneity in the patient sample by studying genetically isolated populations or by narrowing the affected phenotype under study based on criteria of severity or the presence of a biological marker for the disease. Another approach is to greatly expand the sample size and number of DNA markers used in genetic association studies to increase the power to detect multiple possible genes contributing to disease in subsets of the sample. In either case, both pedigree and population-based genetic mapping studies are expected to yield more promising results in the future due in part to the extensive characterization of the human genome provided by the HGP. The HGP, begun in 1990, is a joint effort coordinated by the U.S. Department of Energy and the National Institutes of Health, with the cooperation in recent years of international entities such as the Wellcome Trust in Great Britain (3). One of the HGP's main goals is to finish the complete human genome sequence by the end of 2003 while concomitantly identifying all the estimated 100,000 genes in human DNA and creating the most dense and accurate genetic maps for genome screening studies. The next subsection describes how innovations in genetic maps and the structure of genetic mapping studies may eventually lead us to identify the as yet elusive genes for psychiatric disorders.

Genetic Maps

At the time of this writing, commonly used genetic maps consist mostly of microsatellite DNA markers (usually repeats of two, three, or four nucleotides that vary in length among individuals) that occur with fairly even spacing across the entire genome. These maps contain several thousand markers spaced at roughly 1 to 5 centimorgans (cM); 1 cM is a unit of genetic distance equivalent to a recombination frequency between two loci of 1%, i.e., one recombination would occur per hundred meioses. Much denser maps are under construction now, however, as part of the HGP. In fact, a major goal of the HGP is to characterize the extent of genetic variation that exists among humans in order to create a map of several hundred thousand markers to enable high-density genome screening studies of complex traits (4). Differences in single bases of DNA known as single nucleotide polymorphisms (SNPs) are thought to constitute roughly 90% of sequence variation in humans. Occurring at an average spacing of 1 SNP per 1,000 base pairs (1 kilobase, kb), they will thus constitute the majority of the markers in the planned high-density map. To facilitate identification of these SNPs, the National Institutes of Health (NIH) recently assembled cell lines and DNA from a collection of 450 anonymous, unrelated individuals representing the major ethnic groups of the United States; this collection is known as the DNA Polymorphism Discovery Resource (DPDR) (5). The DPDR is available to investigators to facilitate detection of population genetic variation in their loci of interest, with the expectation that they will share this information with the scientific community. For instance, SNP variants in the coding or regulatory regions of genes (cSNPs) may cause functional differences in gene expression. With such variants catalogued in advance, it will be relatively straightforward to test multiple candidate genes for association with a disease phenotype or a pharmacogenetic effect. The enormous task of identifying and scoring SNPs in large samples, or performing the projected high-density genome screening studies, has necessitated the development of high-throughput technologies such as DNA chips (6), which are discussed elsewhere in this volume.

Principles of Genetic Mapping: The Search for Identity by Descent

Genetic mapping methods are based on the expectation that a proportion of patients in a study population, whether members of an extended pedigree or more remotely related descendants of a common ancestor, will share segments of DNA identically by descent (IBD) in the vicinity of the disease gene under study. The principle behind this expectation is best illustrated by considering genetically isolated or founder populations (7,8). A founder population descends from a small number of ancestral individuals and grows in relative isolation with little admixture, so it is genetically homogeneous. The premise is that a disease mutation is introduced into a population on a particular "founder" chromosome, which is then transmitted to patients descended from a common ancestor carrying this mutation. Over subsequent generations, recombination will reduce the size of the segment of DNA that patients share around the disease gene. The shared segment includes the markers that flank the disease gene. These markers are said to be linked to the gene since, because of their close physical proximity, they seldom recombine with each other and hence are transmitted as a unit or segment. Detection of linkage is the goal of pedigree-based genetic studies. Alternatively, if alleles at markers co-occur more frequently than expected given the known allele frequencies and recombination fraction between the markers, they are said to be in linkage disequilibrium (LD). Evidence of LD between markers also indicates that they are probably close to each other. If LD is observed between the same markers over a region greater than occurs at random in a sample of patients, it may indicate that they share this segment of DNA IBD and that it harbors a disease gene. Detection of LD is a goal of population-based genetic studies.

The length of the IBD segment around a disease gene is inversely proportional to the number of generations by which patients are separated from a common ancestor (Fig. 18.1), and thus genomic screening strategies to detect such a segment will depend on the "age" of the study population (9). For example, a sample of affected individuals separated by roughly 15 generations from their common ancestors

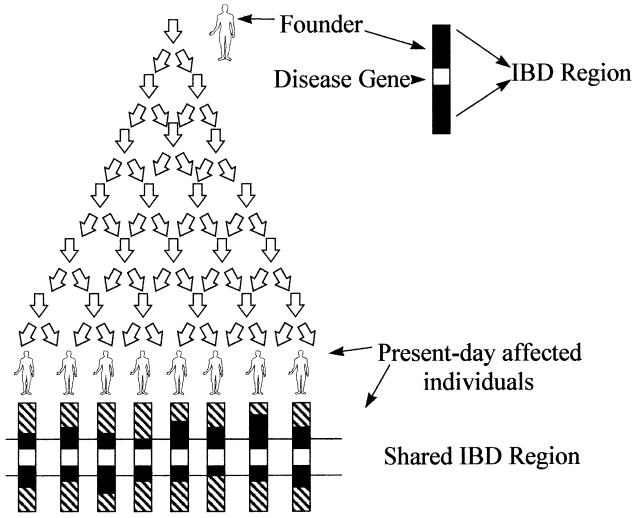

FIGURE 18.1. Genetic mapping studies in isolated populations take advantage of the fact that many recombination events separate affected individuals in the present day from a common disease ancestor. As a result, the majority of patients should share a segment of DNA around the disease gene that is longer than any other DNA segment they might share by chance, but still small enough for positional cloning purposes.

might be expected to share segments around a disease gene detectable in genome screens using current microsatellite maps with markers spaced at 3 to 5 cM. In contrast, the Finnish population is a founder population in which present-day individuals are separated from their common ancestors by up to 100 generations. In this case, shared DNA segments harboring a particular disease gene may be so small that one would have to screen the genome with a much denser marker map (e.g., markers every 0.1 cM) to find them. Such screening studies in a nonhomogeneous population such as that of the United States, wherein common ancestors must be located in the very remote past, will require use of the planned SNP map of around several hundred thousand markers in order to detect regions of LD, which, it has been hypothesized, may be as small as 3 kb (10).

Alternatively, identification of much larger chromosomal regions that are IBD among a sample of patients may also be carried out in extended pedigrees wherein the small number of meioses separating affected individuals leads to a greater length of IBD sharing around the disease gene. Such regions may be easier to detect in pedigrees with genome screens using markers spaced at broader genetic intervals (on the order of 5 to 10 cM). It can be difficult, however, to find recombinant individuals that will allow refinement of the candidate interval to a sufficiently small region to facilitate positional cloning. We review the relative strengths and weaknesses of pedigree- and population-based genetic studies below.

Pedigree-Based Mapping Studies: Problems and Solutions

Linkage analysis of pedigree data has been a very successful method of mapping genes for rare single gene disorders with distinct phenotypes such as cystic fibrosis (11). There are many limitations on the use of linkage analysis for complex traits, however. Linkage analysis is a statistical means of quantifying the likelihood that the observed segregation of marker alleles within a family supports the hypothesis of linkage versus nonlinkage to a disease gene. Traditional linkage analysis is model-based or parametric, meaning that it requires specification of disease inheritance parameters that are not easily estimated for psychiatric disorders, such as the frequency of the disease allele, the genotype specific penetrance, or even the number of genes likely involved. The lack of knowledge of these parameters means that data must be analyzed under a number of different models. This process of multiple testing diminishes the strength of statistical conclusions such that it may be nearly impossible to distinguish between background "noise" and true but weak signals coming from multiple genes of small effect (12). However, several strategies have been successfully implemented to increase the power of linkage analysis for complex nonpsychiatric illnesses by attempting to reduce genetic heterogeneity in the patient sample or by using methods of statistical analysis that do not require specification of genetic model parameters.

Solution 1: Reduce Genetic Heterogeneity in the Sample

Strategies for reducing genetic heterogeneity include studying a small number of large, multiply affected pedigrees ascertained, if possible, from a founder population and narrowly defining the phenotype understudy. The premise in the former case is that the number of genes contributing to a particular disease phenotype within one or a few large families may be less than in many small families or the population at large. This premise is also more likely to hold if the number of disease loci in the population as a whole is limited, as may be the case for founder populations (7, 8). Furthermore, a multiply affected family may indicate that the gene or genes involved are highly penetrant (penetrance refers to the likelihood that a person who has a disease gene will manifest the disease phenotype) and may be easier to find.

For nonpsychiatric complex traits, refinement of the affected phenotype can be accomplished in several ways. For instance, limiting the affected phenotype to include only the most extreme or distinct form of the illness under study has also been critical to the success of mapping studies for complex traits, as such phenotypes are expected to reflect a more homogeneous genetic etiology than more broadly defined phenotypes (13,14). Illustrating these points, a gene

for a severe form of Alzheimer's disease (AD) characterized by early age of onset was detected in a few large multiply affected pedigrees ascertained from a genetically isolated population of German descent (15,16). Although such a gene may not contribute significantly to AD in the general population, it may still provide clues as to relevant biological pathways that might suggest candidate genes for other mapping studies.

Another way to refine an affected phenotype is to require the presence of an objective measure associated with the disorder such as elevated immunoglobulin E (IgE) levels in patients with asthma (17). However, we still have no comparable biological markers for psychiatric disorders at least when these disorders are defined by current nosology. Hence, investigators are attempting to find endophenotypes or subcomponents within psychiatric syndromes that may be objectively measured and inherited in a more straightforward fashion than the constellation of symptoms that constitute the full psychiatric syndrome (18–20). What differentiates this strategy from other attempts to refine traditional psychiatric phenotypes is that family members who have not received a psychiatric diagnosis may still be segregating the trait of interest and may display an endophenotype that allows them to be considered "affected" for genetic study.

Many of the efforts to investigate familial transmission of endophenotypes have focused on schizophrenia. For example, investigators have hypothesized that abnormalities of sensory gating are biological markers for attentional dysfunction, which seems to be a core phenotypic characteristic of schizophrenia or psychosis. Abnormal ocular movements and failure to suppress evoked responses to auditory stimuli after a cue (the P50 response) are both thought to be transmitted within families of schizophrenic probands whether or not family members have a psychiatric diagnosis (21–25). Two studies have examined the same set of families for linkage to either an abnormal P50 response alone or in combination with abnormal oculomotor movements (26, 27). Evidence for linkage to each phenotype implicated different loci (the α_7-nicotinic acetylcholine receptor subunit gene and a region in chromosome 22q11-12, respectively) and was stronger than evidence for linkage of the schizophrenia phenotype alone.

Few endophenotypes have been characterized so far for mood disorders; however, a possible endophenotype is that of suicide (28). Roy et al. (29) studied the monozygotic and dizygotic co-twins of twin suicide victims and found that significantly more monozygotic co-twins than dizygotic co-twins also attempted suicide, possibly arguing for a genetic component to this behavior. Investigators are continuing to develop brain banks of suicide victims for postmortem studies including genetic screens and searching for relevant biochemical markers (30).

Solution 2: Alternative Analytic Models

As an alternative to model-based methods, nonparametric or "model-free" methods can be utilized to detect linkage and may be more robust when the true mode of inheritance is unknown. These methods were originally developed for samples of affected sibling pairs but have now been modified for analysis of other types of relative pairs or whole pedigrees. Simply stated, nonparametric methods are designed to calculate the amount of IBD sharing of marker alleles among affected relatives where the null hypothesis is that transmission of alleles is independent of transmission of disease. For any pair of affected relatives, the probability that the pair will share zero, one, or two alleles IBD can be calculated based on their degree of relationship. Linkage is detected if the sharing of marker alleles among affected relatives is increased over the sharing expected given their relationship.

Demonstration that patients share a series of alleles over multiple markers on the same chromosome, also known as haplotypes, can definitively establish that a segment of DNA has been inherited IBD and thus likely harbors a disease gene. Methods that can quantitate the significance of haplotype sharing among affected individuals are thus particularly useful tools for determining candidate gene intervals in both pedigree and population samples, although the inheritance patterns within extended pedigrees need to be fully characterized to avoid misinterpretation of the allele sharing data.

In addition to the problems with statistical detection of linkage, another major shortcoming of pedigree and affected relative pair studies is that investigators may detect a disease gene but be able to localize it only to a very broad genetic interval. The extent to which a genetic interval containing the disease locus can be narrowed to a small-enough interval for positional cloning purposes depends on the number of opportunities for recombination of the disease haplotype in affected persons; in pedigrees where individuals are separated by only two or three generations, opportunities for recombination of the disease haplotype are limited. Although affected relative pairs are usually much easier to collect than multiply affected pedigrees, very large numbers are required to detect linkage, and the accuracy of gene localization is usually much less than that provided by pedigrees.

Finally, when studying complex traits it is very likely that some individuals will be phenocopies, which means that they exhibit the phenotype under study but due to different genetic or environmental factors. In this case, one may be misled by apparent recombination events even in a single individual, and may therefore incorrectly delineate the candidate interval for a disease gene in a region.

All of these factors can seriously impede localization and positional cloning efforts for disease genes in pedigree samples.

Population-Based Mapping Studies

Given the limitations on pedigree studies described above, analysis of a population-based sample is frequently a preferred strategy for high-resolution mapping of disease loci (31). One reason for this is that many meioses (and therefore opportunities for recombination) have occurred since the patients in such a sample were separated from their common ancestor, so there is a better chance to narrowly define a candidate gene interval (Fig. 18.1). Risch and Merikangas (32) also proposed that population mapping strategies might be a more efficient means of initially localizing disease genes (given a sufficient sample size and an appropriately dense marker map), particularly for loci of relatively small effect, as the sample sizes needed for affected relative pair strategies may be huge and thus not feasible.

Association studies are designed to be case-control or family based (see ref. 33 for review). In case-control association studies, allele frequencies at a particular marker are compared between a sample of patients and a sample of controls matched as closely as possible to cases in terms of ethnicity, age, gender, and other relevant socioeconomic variables. Unfortunately, perfect matching can never be guaranteed, and unknown population stratification can occur if many of the cases or controls share an uninvestigated variable. In this setting, the alleles of cases might appear to differ markedly from controls at a particular genetic locus because of such an unknown variable and not because of the presence of the disease phenotype; this could lead to a false-positive result. Such stratification can occur even within distinct ethnic groups. For example, an association study of type 2 diabetes mellitus in a Native-American tribe seemed to indicate that a particular allele of the immunoglobulin complex was protective against diabetes (34). However, after extensive genealogic examination of Native Americans with this allele, it appeared that they all had distant Caucasian relatives. As the allegedly associated allele was common in Caucasians, and diabetes was less common in Caucasians than in the tribe studied, overrepresentation of this allele in nondiabetic Native Americans reflected only the presence of Caucasian admixture and not a true protective effect from diabetes.

Alternatively, affected individuals and their parents are ascertained for family-based studies that utilize the alleles on the nontransmitted chromosomes of parents as controls for the patients' alleles to prevent ethnic mismatching. One commonly used approach to analyzing such family data is the transmission disequilibrium test (TDT) (35). In this test each allele of a heterozygous parent is measured to see if it is transmitted to an affected offspring significantly more often than the expected 50% by chance. In this case, the implicated allele would be both associated and linked to the disorder, obviating the possibility that the allele is falsely associated through population stratification. Other approaches for analyzing family-based association data use nontransmitted parental alleles as controls, but do not evaluate actual transmission of these alleles to the patient and do not exclude data from homozygous parents. One such approach is the likelihood-based method of Terwilleger (36). A disadvantage of family-based LD methods is that it can often be difficult to sample parents of affected individuals, especially for adult-onset disease.

The LD tests described above are often used to examine the association at single markers individually, which can also be problematic because a very large number of markers must be used for LD genome screening studies, even in isolated populations, and statistical correction for multiple testing is necessary. Interpreting the significance of single-point association tests in this setting becomes extremely difficult (37). Fortunately, the development of multipoint statistical methods for quantifying the significance of haplotypes shared over multiple markers could help to increase the power to detect even weak LD signals coming from a subset of the sample. Such approaches are inherently more powerful than single-point tests of association and will be essential for the evaluation of data generated from SNP maps.

One promising LD method, termed ancestral haplotype reconstruction (AHR), assesses the likelihood that a sample of patient haplotypes have descended from a common mutation-bearing founder haplotype (38). This method is currently being modified so that it will be useful both for genome screening and subsequent fine-mapping studies. At the genome screening stage, markers are generally spaced at sufficient distances such that they can be considered to be in linkage equilibrium with each other in distantly related affected persons. Detecting LD between two or more markers in this setting should thus point to the candidate gene interval as long as the underlying assumption is met that the markers tested are not in LD with each other independent of the disease phenotype (so-called background or random LD); it is still not certain how such background LD is distributed within the genome and between different populations. Once a candidate region has been identified by LD analysis, the next step is to type as many markers as closely spaced as possible within the area to determine the minimal interval of maximal IBD sharing that should contain the disease gene. Although this step can be accomplished in some cases just by observation (39), a statistical method that can assign some level of significance to the observed data would be very useful, especially if the disease haplotype is relatively common and a large sample is required to detect its contribution to the phenotype. However, multipoint analysis of markers typed at high density for fine mapping (or for genome screens with dense SNP maps) is complicated because, since the markers are so closely spaced, one cannot assume that these markers are not in LD with each other independent of the disease phenotype. Multipoint LD methods such as AHR will need to take into account the possibility that significant background LD could occur be-

tween closely spaced markers in order to distinguish this background LD from what may be a very subtle increment of LD surrounding the true disease locus.

In summary, the ability to localize disease genes using LD methods in a given population sample depends on the amount and extent of LD present, the number of disease predisposing alleles at a given locus (allelic complexity), the degree to which the disease locus increases the likelihood of manifesting the affected phenotype, and the power of current statistical methods to measure existing LD. For an excellent review of the strengths and weaknesses of current statistical approaches for analyzing LD, see ref. 40.

Identification of a Disease Gene

Should psychiatric geneticists overcome the many obstacles facing them and succeed in mapping a disease locus to a specific interval, the next step would be to identify the disease gene within it, a process termed *positional cloning* or, given the completion of genome sequencing, the *positional candidate approach* (41). Positional cloning in its purest sense is the process of identifying a disease gene based only on knowledge of its chromosomal location as determined by linkage analysis or LD mapping, without any knowledge of the gene's function. This process involves laborious efforts to build a physical map and sequence the region. Physical maps are made by isolating and linking together yeast and/or bacterial artificial chromosomes (YACs, BACs) containing fragments of human DNA from the region. These fragments are then sequenced and ordered so that the genomic DNA sequence across the candidate gene region is known. Then comes the arduous process of identifying all the genes in the interval and performing mutation detection. Advances in physical mapping and gene-finding technologies arising from the HGP have greatly speeded up this process, however. For instance, near-complete genomic sequence data for the region of interest may already have been deposited in the public databases, obviating the need for extensive genomic sequencing. Next, an investigator may explore large databases of partial complementary DNA (cDNA) sequences also known as "expressed sequence tags" (ESTs) to find most or all of the genes mapping within the area. In addition, there are already a multitude of Web-based sequence analysis programs that provide a host of information such as exon and promotor prediction, open reading frames, and protein homologies for translated sequence. This computer exercise is part of the positional candidate approach. Once all the ESTs have been identified, the complete sequence of the corresponding genes (including definition of intron/exon boundaries and the promotor, if possible) are elucidated and mutation detection begins. If the causative mutation is not detected in coding sequence, however, it can be very difficult to detect disease-causing mutations in the surrounding noncoding DNA or introns, as these regions are large and likely to display more natural variation than highly conserved coding sequence. One possible strategy in this situation is to search for an orthologous (similar) gene in a model organism such as the mouse. Comparison of these sequences may highlight strongly conserved regions of DNA outside of coding sequence that may be functionally relevant and important to examine closely for disease predisposing variants (42). Provision of the complete sequences of the genomes of model organisms amenable to genetic manipulations such as flies, mice, roundworms, and yeast by the HGP could speed understanding of comparable gene structure and function in humans and serve as a molecular confirmation and/or supplement to current gene prediction programs (43,44).

PHARMACOGENOMICS

As genes contributing to the development of psychiatric disorders are discovered, they will be added to the known array of neurotransmitters, receptors, and transporters that are already considered candidate genes for pharmacogenetic analysis. Pharmacogenetics is the study of the genetic mechanisms determining an individual's responsiveness to drugs (45). In this approach, genes involved in drug metabolic pathways and sites of action, or disease processes if known, are examined for naturally occurring variants or polymorphisms, which may then be shown to affect the expression of that gene. This effect on gene function may then be linked to the efficacy of the drug and/or a predisposition to particular side effects in individuals with that genotype. For example, the apolipoprotein (apo) E4 allele at the apo E locus has been shown to be associated with late-onset AD, as well as to a poor response to cholinesterase inhibitor treatment of AD (46). The downside of this strategy is that it is limited by the paucity of candidate genes with proven association to psychiatric phenotypes. Fortunately, investigators in the emergent field of pharmacogenomics are seeking to identify previously unsuspected genetic markers of drug responsiveness by using a variety of high-throughput genomics technologies to examine drug-induced changes of gene expression in different tissues throughout the body. We provide here a brief overview of the principles of pharmacogenetics and pharmacogenomics (see ref 47. for review), focusing on how these approaches are being applied in an effort to provide a more rational basis for pharmacotherapy of psychiatric disorders than the trial-and-error approach we currently employ.

The impact of genetic variation on drug response is characterized broadly by changes in pharmacokinetic and pharmacodynamic parameters. Pharmacokinetic studies assess the processes of absorption, distribution, first-pass and general metabolism, and elimination of drugs. Transport processes in renal, intestinal, and hepatic epithelia and drug metabolizing enzymes exhibit genetic variability, which will in many cases be likely to influence the pharmokinetics of

relevant drugs. In the latter situation, the cytochrome P-450 system has been best studied (48) beginning with the classic example of the poor metabolism of the antihypertensive drug debrisoquine due to several mutant alleles of the polymorphic CYP2D6 gene also known as debrisoquine/sparteine hydroxylase. This enzyme is responsible for the metabolism of roughly a quarter of all drugs including most antipsychotics and antidepressants (49). This enzyme is also inhibited potently by fluoxetine. About 7% of Caucasians and an even greater percentage of Asians are poor metabolizers of such drugs due to polymorphisms in this enzyme. On the other hand, some persons carry different alleles and/or multiple copies of this gene, which predispose to more rapid metabolism; up to 13 copies have been documented in a single individual. A study of nortriptyline metabolism in these individuals clearly demonstrated that clearance of nortriptyline was proportional to the number of copies of the *CYP2D6* gene, especially the CYP2D6*2 allelic form (50,51). Knowledge that a person has a genotype predisposing to unusually slow or rapid metabolism could guide appropriate drug choice and dosing regimen. Unfortunately, despite intensive study, no definitive relationship between polymorphisms in cytochrome P-450 enzymes and drug efficacy or predisposition to side effects of antidepressant drugs has yet been discovered (52).

Pharmacodynamics concerns the relationship between the concentration of a drug and response at its site of action, for example at receptors and transporters for neurotransmitters. Pharmacodynamic effects may also vary temporally, and so both the acute and chronic nature of response to the drug must be considered. In the acute phase of responsiveness, receptor polymorphisms could alter any of the myriad steps in a pathway from receptor-drug binding through the cascade of signals that result; such variants may determine who is more prone to immediate drug reactions, for example the malignant hyperthermia that may occur in response to antipsychotics. Genetic variation could also play a role in the drug-induced neural plasticity that occurs as a result of the chronic treatment required for alleviation of psychiatric symptomatology as well as chronic use of addictive substances. Adaptive responses to drugs will vary among individuals, and genetic factors may predict such phenomena as waning of drug response over time, and proneness to side effects such as tardive dyskinesia induced by antipsychotics (53).

From a pharmacogenetic perspective, one of the most obvious candidates for studying psychiatric drug responsiveness identified to date is the serotonin (5-hydroxytryptamine, 5-HT) transporter (5-HTT) (52). This transporter plays a critical role in the termination of serotoninergic transmission and is the target of the most widely prescribed family of antidepressants, known collectively as the selective serotonin reuptake inhibitors (SSRIs). Heils et al. (54) identified in 1996 a variant of 5-HTT with a 44–base pair (bp) insertion within the promoter region, which is commonly referred to now as the long (versus the short) allele. Lesch et al. (55) then demonstrated that baseline levels of transcription of the long variant were more than double that of the short variant in transfected cells and that this difference was reflected in altered 5-HTT expression and 5-HT reuptake). Since then, investigators have studied depressed patients to see if they can correlate SSRI response with alleles at the 5-HTT promoter. Smeraldi et al. (56) presented data suggesting that patients with delusional depression responded better to fluvoxamine if they were homozygous for the long allele of the 5-HTT promoter. Other investigators have obtained similar findings in a samples of depressed patients treated with paroxetine (57), although in this study rapidity of response was improved in persons homozygous for the long allele, while overall outcome at 12 weeks was the same for all genotypes.

Besides the serotonin transporter, there are several thousand other genes for neuromodulatory molecules that have been cloned and expressed in some type of cellular system and that are viable candidates for drug targets (58). Pharmacogenomic technologies may aid in prioritizing these candidates for examination, or identifying yet more candidates, by determining those genes that are activated or deactivated in tissues during an acute psychiatric episode and in response to treatment. One approach to evaluating gene expression involves hybridization of fluorescent or radioactively labeled messenger RNA (mRNA) samples taken from the relevant cell population to cDNA arrays. Changes in gene expression (up, down, or none) can then be compared between different samples at a single time point or within a sample overtime. This technique is known as serial analysis of gene expression (SAGE) (59). However, if the changes in gene activation induced by disease or by drugs are localized to a specific population of cells in inaccessible tissues such as that of the brain, rather than, say, in fibroblasts from skin biopsies, the SAGE technique will not be helpful. Alternatively, large-scale analysis of proteins within clinical samples is also predicted by some to become a useful means of identifying biological markers indicative of a response to drugs (60). Again, any changes in protein expression would need to be detectable in easily obtainable fluids such as blood or urine to be of use in the evaluation of psychiatric disorders, and the process of informed consent for such experimentation would need to be reviewed thoroughly.

SUMMARY

Genomics has great potential to advance the field of psychiatry in general and neuropsychopharmacology in particular. This will occur through the identification of genes involved in the etiology of psychiatric disorders and the identification and characterization of genes involved in the response to psychotropic agents. Although we have not made much headway as of yet in either of these fields, impressive ad-

vances in our knowledge of the genomes of human and model organisms as well as access to the technologies developed to provide this information are likely to stimulate swift progress. Success in these endeavors will lead to more efficient identification and development of drug targets, the provision of an objective basis for choice of drug, ''custom drugs'' based on an individuals genotype, improved diagnosis of disease, and earlier detection of genetic predispositions to disease.

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