Part I

Introduction

1

Genes and psychopharmacology: exploring the interface

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OVERVIEW

Pharmacogenetics is the study of genetically determined, interindividual differences in therapeutic response to drugs and susceptibility to adverse effects. The principal objective of pharmacogenetics is to identify and categorize the genetic factors that underlie these differences and to apply these observations in the clinic. Individualization of drug treatment to the specific patient is thus a core objective of pharmacogenetics. The goal of this book is to provide a basic conceptual framework for the pharmacogenetics of psychotropic drugs, to address major issues in the design and implementation of studies that seek to advance the field and to provide an overview of findings that have emerged so far. In this introductory chapter, the rationale for psychopharmacogenetics is considered, a brief historical perspective is provided, some of the pivotal concepts and terms are defined, important issues in the design and interpretation of pharmacogenetic studies in psychiatry are considered and optimistic predictions for the future are evaluated. The chapter concludes with a brief overview introducing the reader to the various sections of the book.

Introduction

For as long as medicine has been practiced, physicians have known that patients respond differently to the therapeutic agents that they are administered, even though there are no obvious differences in the nature or severity of their illnesses. Therefore, individual or illness characteristics that might aid the physician in choosing an appropriate treatment have long been sought. The principal objective of pharmacogenetics is to identify and categorize the genetic factors that underlie differences among individuals in their response to drugs and to apply these observations in the clinic.

The pharmacological treatment of psychiatric disorders has made rapid progress since the 1950s. Psychotropic drugs are among the most widely used pharmacological agents worldwide and their number has increased exponentially. These developments have occurred in spite of the highly complex clinical characteristics of

psychiatric disorders and the absence of biological anchors for their diagnosis. Both these limitations are a consequence of the fact that the pathophysiological basis of most psychiatric disorders has not yet been defined. A role for genetic factors in the pathogenesis of many of the major psychiatric syndromes is well established. The advent of modern molecular techniques has led to an intensive search for susceptibility genes, efforts that have yielded intriguing leads but no definitive findings.

This book is positioned at the rapidly developing interface between molecular genetics and psychopharmacology. This area is closely related to the search for susceptibility genes but is also separate from it since genes that affect the therapeutic and adverse effects of the drugs used to treat an illness need not be involved in the pathogenesis of the illness. It is an exciting and increasingly productive interface that holds considerable promise. At the same time, the difficulties and complexities are clearly apparent and will grow more evident as optimistic predictions are submitted to empirical testing. The field holds exceptional fascination and it is still a wide-open frontier in that some of the most fundamental studies have yet to be conducted.

The area of basic concepts, experimental approaches and current findings is too large to be comprehensively addressed in a single volume. Nevertheless, there is a very great need to provide the researcher and clinician with an overview of the pivotal topics and to do so in an integrative way. This chapter is intended to serve as a general introduction to psychopharmacogenetics and to the topics covered in this book.

The rationale for psychopharmacogenetics

Whether medicine is an art or a science may seem an outdated, if not naive, debate at the dawn of the twenty-first century. This impression is bolstered by the extensive basis of modern medicine in biomedical science. Powerful diagnostic technology has immeasurably increased the precision of diagnosis, and evidence-based prescription is rapidly becoming a hallowed cornerstone of therapeutics. Yet, one needs to look no further than pharmacotherapy in order to realize how tenuous the scientific roots of our discipline still are. This is particularly true of the pharmacotherapy of psychiatric disorders but is by no means limited to this field. The psychiatrist who initiates drug treatment of a depressed patient is faced with a bewildering set of choices – at least five classes of drug if one applies current definitions, including tricyclic antidepressants, specific serotonin reuptake inhibitors, monoamine oxidase inhibitors and newer agents that specifically inhibit norepinephrine uptake or enhance synaptic availability of both norepinephrine and serotonin (5-hydroxytryptamine (5-HT)) by mechanisms other than reuptake. Within these classes there are subclasses and within each subclass individual drugs that differ from each other in the intensity or specificity of their pharmacological effects. The

psychiatrist has no definitive way of knowing which patient will respond to which drug. There is very little hard evidence on which to base a rational choice, certainly not in the area of therapeutic efficacy. Usually the choice is made on the basis of adverse effect profiles or clinical experience.

The situation described is by no means limited to psychopharmacology. The neurologist treating epilepsy and the internist or pediatrician treating asthma (notwithstanding recent advances in the pharmacogenetics of this disorder) are in the similar position of having to make educated guesses regarding the choice of medication on the basis of a far from comprehensive set of evidence-based criteria. This situation is an inevitable consequence of the fact that modern, evidence-based therapeutics is by definition group and not individual oriented. Particularly in psychiatry, large numbers of patients are required to demonstrate unequivocally a significant drug–placebo difference, and drug–placebo differences are the cornerstone of evidence-based medicine. In cases where a drug is effective in two thirds of subjects (versus one third of subjects for placebo), efficacy may be unequivocally demonstrated, but a less appreciated element of the message is that one in three patients will not respond. Moreover, among responders to the active drug, at least one third might be placebo responders.

It is envisaged that pharmacogenetic predictors of response to drugs and of adverse effects will ultimately serve as the basis for simple diagnostic tests that can be applied in the clinic in order to prescribe the appropriate drug to the appropriate patient. In the current era of managed care in medicine and limited resources, this will be a development of major economic importance. As discussed in this chapter, the obstacles on the way to this objective are formidable but not insurmountable. Another very important application of pharmacogenetic screening will be in clinical trials. Knowledge of genetic predictors of response and/or adverse effects, even if this is not at a level of resolution that permits applicability in regular clinical practice, will permit stratification of patients in clinical trials. This will substantially reduce the cost of drug development and shorten the lengthy lag period that currently elapses until a drug is introduced into the clinic.

With the publication of the draft sequence of the human genome, it has become accepted practice to refer to the current era as "postgenomic." In this postgenomic era, pharmacogenetics, a discipline with a long and distinguished "pregenomic" history, has come of age. Powerful tools have been placed at its disposal and optimism abounds as to its anticipated impact on pharmacotherapy.

Historical perspective

Nebert (1997) suggests that Pythagoras was the first to recognize the basic principle of pharmacogenetics when, around 510 BC, he noted the predisposition of

some individuals but not others to develop an adverse reaction (hemolytic anemia owing to glucose 6-phosphate dehydrogenase deficiency) after consumption of the fava bean. A century ago, the English physician Archibald Garrod suggested that genetic factors directed chemical transformations in humans. His ideas on the hereditary basis of chemical individuality are extensively discussed in his writings (Garrod, 1902, 1909). A seminal study was that of Snyder (1932), which defined the phenylthiourea nontasting phenomenon as an autosomal recessive trait. According to Propping and Nothen (1995), the relationship between adverse drug reactions and genetically determined variation was first demonstrated by Motulsky (1957). The term "pharmacogenetics" was coined by Vogel in 1959. Another important development was the observation that the antitubercular drug isoniazid could be slowly or rapidly acetylated and that this was under genetic control (Evans et al., 1960). A further milestone was the demonstration by Kalow of an abnormal form of serum cholinesterase that leads to catastrophic adverse reactions to succinylcholine. Kalow also wrote the first systematic account of pharmacogenetics (Kalow, 1962). Polymorphism of the P-450 enzyme now termed CYP2D6 was first observed in the 1970s in healthy volunteers who developed adverse effects when taking the antihypertensive agent debrisoquine. The enzyme was initially named debrisoquine hydroxylase but it was subsequently shown that oxidation of sparteine is by the same enzyme (Mahgoub et al., 1977; Eichelbaum et al., 1979).

For much of its history, the focus of pharmacogenetics has been on drug-metabolizing enzymes. This was because of the availability of techniques to detect phenotypic differences between individuals in the plasma level of drugs and to study their genetic basis. The focus on pharmacodynamic variation is more recent and was given considerable impetus by the advent of techniques to determine the sequence of genes and identify variations.

Definition of terms

Table 1.1 summarizes many of the terms that will be used frequently throughout this book. While *pharmacogenetics* is defined as the study of genetically determined interindividual differences in response to drugs, *pharmacogenomics* refers to the use of genome-based technologies in drug development. The fields are closely related and the terms are often used interchangeably. Nevertheless, it is useful to maintain the distinction because of the different starting points and outcomes. Pharmacogenetics is individual based and its body of information is derived from the relationship between drug effects and genetic predisposition in patients or volunteer subjects. Its outcome is rational drug choice in the treatment of patients, based on the evidence that has accumulated. The starting point of

Table 1.1. A pharmacogenetics glossary

Term	Definition
Pharmacogenetics	The study of genetically determined interindividual differences in response to drugs
Pharmacogenomics	The use of genome-based technologies in drug development
Types of pharmacogenetic effects	
Pharmacokinetic	Genetically based differences that influence bioavailability of a drug
Pharmacodynamic	Genetically based differences in the proteins at which a drug acts
Polymorphism	Genetic variation that occurs with a frequency of 1% or more in the population
Single nucleotide polymorphisms (SNPs)	Differences between individuals in a single base of the genomic sequence; these are the most frequently occurring genetic variation
Coding	Occur in exons (coding regions) of genes
Noncoding	Occur in introns (noncoding regions) of genes
Regulatory	Occur in 3′ or 5′ regulatory regions (promoter)
Synonymous	SNPs in coding regions that do not influence the structure of the protein
Conservative	Alter the structure of the protein but not its function
Functional	Alter the function of the protein
Candidate gene	Chosen for analysis on the basis of an a priori hypothesis regarding the role of the protein coded by the gene in the phenotype under study
Association	Statistical demonstration of a greater than chance occurrence of a polymorphism in a candidate gene in conjunction with the target phenotype
Linkage disequilibrium (LD)	Statistical association of two alleles at a rate greater than would be predicted by chance (owing to the fact that the two alleles are close enough to each other to not undergo recombination)
Polygenic	A trait that is influenced by a number of different genes each of which contributes a portion of the effect
Epistasis	Genetic variance owing to nonadditive effects of alleles at distinct loci
Multifactorial	Both genetic and environmental factors contribute substantially and variably to the phenotype

pharmacogenomics is the human genome sequence and its outcome is the development of new pharmacological agents. The points of interaction between the two approaches are multiple and they complement each other at many levels, so it is inevitable that the distinction is easily blurred.

There are two broad categories within pharmacogenetics, which derive from the fact that differences between individuals can be attributed to two major factors that are under genetic influence. The first set of factors is *pharmacokinetic* and they encompass genetically based differences in processes that influence bioavailability of a drug, i.e., the concentration of the drug and its active metabolites that is available at the site of action). The second major category is *pharmacodynamics* and refers to genetically based differences in the proteins at which the drug acts. Both sets of factors may influence the response of the individual to a given drug and they may interact within the same individual.

Polymorphism is a core term in pharmacogenetics. A polymorphism is a genetic variation that occurs with a frequency of 1% or more in the population. Genetic variations that occur more rarely (mutations) also influence drug response, often dramatically, but these are of less importance on a population-wide basis. *Single nucleotide polymorphisms* (*SNPs*) are differences between individuals in a single base of the genomic sequence and are the most frequently occurring genetic variation. SNPs occur throughout the human genome at a density of approximately 1 per 1000 bases (kilobases, kb) of DNA. It is important to stress that the vast majority of SNPs are unlikely to influence either the structure or function of proteins. SNPs may be classified by their location, occurring in exons (coding regions) or introns (noncoding regions) of genes and in regulatory regions such as the promoter. SNPs in coding regions need not necessarily influence the structure of the protein and are termed synonymous. SNPs that do alter the structure of the proteins need not have functional consequences and are termed conservative or non-functional. SNPs located in intronic regions can have an impact on the coded protein by influencing splicing (Krawezak et al., 1992). SNPs in regulatory regions are a focus of considerable interest and can have major effects on expression of the gene. In fact, it has been suggested that regulatory mutations rather than mutations that affect protein structure may be the prime cause of biological differences in humans (Chakravarti, 1999; Chapter 12). Major efforts are now in progress by public and industry-based consortia to generate SNP databases that will be an invaluable resource for pharmacogenetics in the very near future.

SNPs are not the only type of DNA variation of relevance to pharmacogenetics. Other types of genetic polymorphism result from the insertion or deletion of a few nucleotides (termed *insertion/deletion polymorphisms*) and variation in the number of times a sequence is repeated. *Variable number tandem repeats* (*VNTRs* or

minisatellites) have several hundred base pairs repeated while *microsatellites* (or *simple tandem repeats, STRs*) have two to four nucleotides repeated a variable number of times. Allelic variations (i.e., variability in the number of repeats) can be considerable and this type of variation is consequently highly polymorphic.

Traditionally, pharmacogenetic studies have sought an association between a specific gene and the response or adverse effect phenotype under study. This is a classical *candidate gene* approach, which is based on an a priori hypothesis regarding the role of the protein coded by the gene in the particular phenotype. However, an SNP or other genetic variation may be statistically associated with a phenotype without having a direct effect. This phenomenon results from *linkage disequilibrium* (LD) and it arises from the fact that the variant examined is close enough to the true predisposing variant that it does not undergo recombination during meiosis and is inherited with it. The marker may be at a different site in the gene itself or may be located outside the gene. Linkage disequilibrium mapping employs dense SNP maps in order to localize genes associated with a phenotype and has been suggested as a powerful approach to identify genes for complex phenotypes (Risch, 2000), although there are dissenting voices that question the feasibility of the strategy (Weiss and Terwilliger, 2000). Linkage disequilibrium approaches are the cornerstone of the large-scale mapping projects in pharmacogenetics that are currently being advocated.

Classical genetics of human disease deals with monogenic disorders in which a single mutation in a single gene is causatively related to the phenotype. This paradigm holds reasonably true in pharmacogenetics for classical "pharmacokinetic" polymorphisms that have a major effect on drug bioavailability (such as the effect of CYP2D6 polymorphism on the metabolism of a variety of psychotropic and other drugs, which is inherited as an autosomal recessive trait). For the most part, however, the accepted view is that pharmacogenetic traits are likely to be *polygenic* and *multifactorial*. A polygenic trait is one that is influenced by a number of different genes each of which contributes a portion of the effect and may do so additively as well as interactively (*epistasis*). The term "multifactorial" indicates that both genetic and environmental factors contribute substantially and variably to the phenotype.

Core issues in pharmacogenetic research design

The classical experimental context for determining pharmacogenetic influences on drug response is a comparison of responders and nonresponders to the drug or of individuals who develop adverse effects and those who do not. This appealingly simple case-control design should be readily applicable in psychopharmacology. Data from such studies are amenable to analysis by two approaches. The first is a

categorical approach in which patients are grouped according to the phenotype (responder or nonresponder, develops adverse effect or does not) and the frequency of the genotype of interest in these groups is compared. The second approach utilizes the response variable (or adverse effect measure) in a continuous fashion and compares scores at a single time point or over a period of treatment in patients grouped according to genotype. There are already numerous examples of the application of this approach to psychopharmacology. The paucity of replicable results may be a consequence of a number of relatively elementary factors.

Population effects

It is well known that the frequency of genetic polymorphisms differs markedly among ethnic groups. Therefore, unequal inclusion of individuals of different ethnic backgrounds in groups of subjects being compared can lead to spurious results that reflect ethnic stratification. Methods have been proposed to address these issues (Devlin and Roeder, 1999; Lerer et al., 2001). Nevertheless, it is essential that studies be designed to take them into account prospectively. Another point is that the functional relevance of a particular polymorphism may vary among ethnic groups. Thus, a true finding in a sample from a particular population may not be replicable in a different population.

Demographic variables

Since the basis of interindividual variability in drug response is multifactorial, the impact of demographic variables such as age and gender cannot be ignored. A particular genetic variant may not be functionally relevant without the addition of other factors that also influence the phenotype. An example is the influence of certain 5-HT receptor variants on predisposition to neuroleptic-induced tardive dyskinesia, which was demonstrated in a sample of older patients but was not observed in a sample two decades younger (Segman and Lerer, 2002). It is highly conceivable that gender-specific effects are also operative and that interactions between age and gender will be demonstrated.

Definition and evaluation of the phenotype

Drug response to psychotropic drugs is a phenotype that is very difficult to define for evaluation experimentally. There is an extensive literature that debates how to define a "responder" to an antidepressant drug in the context of a clinical trial. Definitions may be based on percentage improvement on a particular rating scale or by using a specific score on that scale as a threshold. There are conventions that are fairly well accepted for clinical trials of psychopharmacologic agents. It remains to be established whether these conventions can be readily transferred to pharmacogenetic studies (see Rietschel et al., 1999).

Study design

Most recently published studies on the pharmacogenetics of psychotropic drugs are "opportunistic" in that they employ subjects who were previously studied and correlate the phenotypic measures gathered in these studies with genotypic data that are obtained currently. Alternatively, they may be "add-on" in nature. In studies of this type, drawing of blood samples for DNA is included in the protocol of clinical trials for future correlation of genotypes with drug response or adverse effect phenotypes. In both cases, studies are not "purpose designed" to address pharmacogenetic questions. They address the questions that they were designed to answer and do not take into account issues that are pivotal to pharmacogenetics. Since clinical trials demand large sample sizes, there is usually little consideration given to ethnicity of the subjects. The age range may be great and diagnostic boundaries tend to be as wide as possible to insure maximum recruitment. It is likely that even very large samples will not have adequate power to address pharmacogenetic questions when subject groups are stratified in order to control for factors that can spuriously affect the results. A "second generation" of pharmacogenetic studies can be anticipated that are designed to take these issues into account. When this occurs, the issue of placebo control is likely to emerge as a major consideration. On the one hand, it is self-evident that non-placebo-controlled studies greatly impede attempts to differentiate factors associated with "response" from these associated with "response to the active drug." On the other hand, already existing concerns regarding the ethical validity of placebo-controlled trial in disorders such as schizophrenia or depression will be more intense when the aim of the study is refinement of drug prescription rather than demonstration of efficacy. Simulation studies are urgently needed in order to address these issues.

Interaction among multiple loci

Traditionally, pharmacogenetic studies have focused on single genes and on single polymorphisms within these genes. It is becoming clear that this approach is too limited to address the complexity of the situation adequately. Single SNPs may not show an association with treatment effects or with disease susceptibility while combinations do. These may be within a single gene, as demonstrated for the effect of complex haplotypes of SNPs in the coding region and the promoter of the β_2-adrenoreceptor gene on the bronchodilator response to asthma therapy (Drysdale et al., 2000). Interactions (epistasis) may be demonstrated between SNPs in different genes, as recently observed in a study of genetic susceptibility to sporadic breast cancer (Ritchie et al., 2001). In the context of pharmacogenetics, Segman et al. (2002) have observed an interaction between a polymorphism in the gene for cytochrome P17 and the gene for dopamine D_3 receptor that is associated with more neuroleptic-related abnormal involuntary movements in patients who carry both mutant genotypes.