Pharmacogenomics

edited by

Werner Kalow
University of Toronto
Toronto, Ontario, Canada

Urs A. Meyer
University of Basel
Basel, Switzerland

Rachel F. Tyndale
University of Toronto
Toronto, Ontario, Canada

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Preface

The term *pharmacogenomics* is a recent arrival in the literature, but it is a term that anyone interested in pharmacogenetics cannot overlook. The increasing prevalence of the term reflects the progressive transition from genetics to genomics that is taking place in pharmaceutical science. The most famous research in genomics is a worldwide program called the Human Genome Project,* a massive effort that is expected to change the science of human biology, including our understanding of human disease. For anyone concerned with drugs and pharmacology, the arrival of the term *pharmacogenomics* is a reflection of this broad change.

The genome of any organism is a complex structure built of almost innumerable molecules of deoxyribonucleic acid (DNA). Within any chromosome, the structure is a continuum made up of small sections called *genes*. It is interesting to note that some experts have recently questioned the scientific relevance of the subdivision of the genome structure into genes. Historically, this logical and useful subdivision came about through the realization that "one gene forms one protein" (but perhaps "peptide" is more accurate than "protein"). However, there is no question that the components of DNA that are not participating in the

* S Collins et al. New goals for the U.S. Human Genome Project: 1998–2003. Science 282:682–689, 1998.

making of peptides at least play important roles by controlling gene expression, RNA splicing, chromatin domain formation, maintenance of chromosome structure, recombinations, and replications. The genome is more than the sum of the genes.

Current pharmacogenetics research deals mostly with inherited changes of protein structure (e.g., changes of drug-metabolizing enzymes) that affect the fate of and thereby the response to a particular drug. Future work in pharmacogenetics may also emphasize the often complex genetic control of the amounts of enzyme and receptor proteins. Although *pharmacogenetics* and *pharmacogenomics* are sometimes used interchangeably, the latter is understood to deal in addition with the identification of new drug targets. Although genetic diseases are generally rare, it is clear that other, more common diseases are usually associated with several or many genetic changes that cause disease in combination with environmental factors. The identification of such a genetic change means that, at least in some cases, drugs can be found that will reverse such a change or compensate for it.

Pharmacogenetics and pharmacogenomics are overlapping sciences, but in practice, pharmacogenomics introduces an additional element. For example, 20 genetic changes may contribute to cardiovascular disease, but only one or two of these changes may cause the disease in a given person. A given drug may alleviate the disease only in patients who are affected by these one or two changes. In addition, pharmacogenetics tells us that some drugs can be taken with safety only by persons without a particular genetic defect or alteration. Thus, there are two elements that point to the future usefulness of personalized medicine, provided that the genome of the patient can be scrutinized appropriately for the absence or presence of relevant mutations.

The effort of looking for relevant mutations has already led to many technical changes that allow examination of numerous genes in many people. A thorough discussion on pharmacogenomics should include a description of some of these new techniques to test genetic changes. Unfortunately, the knowledge of a genetic change may be useless until the affected protein or peptide is identified, and until the physiological or pathological significance of the protein or peptide is known. Therefore, this volume includes a look at the techniques that may provide that knowledge. The relevant techniques will give rise to masses of data that will be analyzed and organized with the help of a new science called bioinformatics. Thus, this book will provide the reader with a broad perspective on current topics as well as problems and solutions that may arise in pharmacogenomics in the future.

Werner Kalow

Contributors

Leif Bertilsson, Ph.D. Department of Medical Laboratory Sciences and Technology, Division of Clinical Pharmacology at Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden

Andrew P. Boright, M.D., Ph.D. Department of Genetics, University of Toronto, and Hospital for Sick Children, Toronto, Ontario, Canada

Alex Garvin, Ph.D. Division of Pharmacology, Biocenter of the University of Basel, Basel, Switzerland

Denis M. Grant, Ph.D. Orchid BioSciences Inc., Princeton, New Jersey

Larry Haff, Ph.D. Applied Biosystems, Framingham, Massachusetts

Laura Hall Applied Biosystems, Framingham, Massachusetts

Daiga Helmeste Department of Psychiatry, University of California, Irvine, Irvine, California

David L. Hyndman Hitachi Chemical Research Center, Irvine, California

Werner Kalow, M.D. Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

Richard B. Kim, M.D. Department of Medicine and Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee

Greg Landes Genzyme Corporation, Framingham, Massachusetts

Stephen L. Madden, Ph.D. Genzyme Corporation, Framingham, Massachusetts

Urs A. Meyer, M.D. Division of Pharmacology/Neurobiology, Biocenter of the University of Basel, Basel, Switzerland

Glenn A. Miller, Ph.D. Genzyme Corporation, Framingham, Massachusetts

Masato Mitsuhashi, M.D., Ph.D. Hitachi Chemical Research Center and Department of Pathology, University of California, Irvine, Irvine, California

Arno G. Motulsky Department of Medicine and Genetics, University of Washington, Seattle, Washington

Michael S. Phillips, Ph.D. Orchid BioSciences Inc., Princeton, New Jersey

Peter N. Ray, Ph.D. Division of Molecular Diagnostics, Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada

Philip L. Ross, Ph.D. Applied Biosystems, Framingham, Massachusetts

B. Michael Silber, Ph.D. Pharmacogenomics and Clinical Biochemical Measurements Department, Pfizer Global Research and Development, Groton, Connecticut

Mark S. Silverberg, M.D. Department of Medicine, University of Toronto, and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

Katherine A. Siminovitch, M.D. Department of Medicine, University of Toronto, and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

Gisela Sitbon, Ph.D. PGL Professional Genetics Laboratory AB, Uppsala, Sweden

Tracy L. Stockley, Ph.D. Division of Molecular Diagnostics, Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada

Ann-Christine Syvänen, Ph.D. Department of Medical Sciences, Molecular Medicine, Uppsala University, Uppsala, Sweden

Siu Tang Department of Psychiatry, University of California, Irvine, Irvine, California

Glenys Thomson Department of Integrative Biology, University of California, Berkeley, Berkeley, California

Clarence Wang Genzyme Corporation, Framingham, Massachusetts

Wendell W. Weber, Ph.D., M.D. Department of Pharmacology, University of Michigan, Ann Arbor, Michigan

Grant R. Wilkinson, Ph.D. Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee

Frank A. Witzmann, Ph.D. Molecular Anatomy Laboratory, Indiana University–Purdue University, Columbus, Indiana

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Historical Aspects of Pharmacogenetics

Werner Kalow
University of Toronto, Toronto, Ontario, Canada

1 INTRODUCTION

Pharmacogenetics deals with heredity and responses to drugs. It is a branch of science that attempts to explain variability of one or another drug responses, and to search for the genetic basis of such variations or differences. Early on, pharmacogenetics research examined differences between individual subjects, but as it developed, it also became concerned with genetic differences between populations. Although many pharmacogeneticists are primarily concerned with the human species, the science can, in principle, be applied to all subjects on earth, primitive or complex, that are capable of responding to a drug or to an environmental chemical.

Pharmacogenetics represents only one of many genetic responses to environmental impacts [1]. Human variation in pharmacogenetics is similar to human variation in response to foods [2]. For instance, modern salt intake causes members of populations who come from salt-poor areas to develop cardiovascular disease [3]. Populations adjusted to frequent periods of starvation tend to show a high incidence of type 2 diabetes [4]. There are different genetic mechanisms to fight infections. There is a gene conveying resistance to tuberculosis, acting before any immune response sets in [5]. AIDS resistance has been explained in Caucasians but not yet in Africans [6]. Thus, pharmacogenetics is not a unique affair, but let us still look at its development.

2 INITIAL PHASE OF PHARMACOGENETICS

2.1 My Unexpected Dive into Pharmacogenetics: A Personal Story

In Berlin in 1948, there were still incidences of malnutrition. Because of this, there were patients who suffered fatal poisoning from the generally safe, local anesthetic drug procaine. This became my impetus to study the esterase that hydrolyzed procaine [7]. When invited to Philadelphia, I continued these studies with the superior equipment there available to me. I found that the procaine-splitting esterase was butyrylcholinesterase, then called pseudo- or plasmacholinesterase, and I explored a method using UV spectrophotometry that elegantly and precisely indicated the esterase activity [8]. I then transferred to Toronto, where pseudocholinesterase had been discovered and where it was still being investigated. I proposed the use of my new UV method to replace the tedious gasometric method then in place. My proposal seemed acceptable to the responsible biochemist, provided I could demonstrate the efficiency of my method by comparing and testing plasma from patients with known high and low cholinesterase activity. Thus I came to test the esterase of patients known to show abnormal effects of succinylcholine, whose esterase had been designated by a government laboratory as having low activity. I was surprised to see that the cholinesterase activity was not low but that it displayed abnormal kinetics with grossly reduced affinity for its substrate, and thus appeared to be low. This could be explained only by an abnormal enzyme structure, and that could only be genetic; I could prove that point by family investigations [9]. I was excited by this observation of interplay between genetics and the abnormal effect of a drug. I tried to find out whether there were other established examples.

My literature search was successful. There was Waldenstrom's story on porphyria [10]. A major find was a report on hemolysis caused by the antimalarial drug primaquine in some American soldiers in the 1940s [11], later shown to be due to glucose-6-phosphate dehydrogenase (G6PD) deficiency. I became excited by a paper of Motulsky [12] entitled "Drug Reactions, Enzymes, and Biochemical Genetics," sponsored by the Council on Drugs of the American Medical Association. I found a report describing genetic differences of the metabolism of isoniazid [13], a then revolutionary antituberculosis drug. These and several other reports encouraged me to write a book on this new topic of pharmacogenetics [14], citing all examples I was able to find. Pharmacogenetics had become my scientific life blood.

2.2 Pharmacogenetics: A Growing Science

Many pertinent observations came in the following years. For instance, Vesell and Page [15] used twin studies to show genetic control of the metabolism of

several drugs. Von Wartburg et al. [16] described a variant of alcohol dehydrogenase. However, a report from the laboratory of Dr. Robert Smith in London [17] became a milestone in pharmacogenetics. He described the deficient metabolism of debrisoquine, a deficiency he had personally experienced as a life-threatening drop of blood pressure after taking the drug [18]. Before that, Eichelbaum had reported in 1975 [19] in a thesis a metabolic deficiency of sparteine metabolism; both defects are now known to be due to deficiency of the P450 cytochrome CYP2D6. This deficiency affects the metabolism of more than 40 drugs; whether the deficiency is clinically important for any given drug depends on a number of drug-associated criteria and safety factors [20]. More than 70 different variants of CYP2D6 are known, many are completely without any trace of activity [21]. On the other hand, gene duplication or multiplication in some subjects causes extremely high CYP2D6 activity [22]. A recent Medline search quoted 1244 papers dealing with CYP2D6 variation. CYP2D6 is the most variable of human CYPs, but its variability is not unique: looking at 10 different CYPs, various observers found 144 alleles and 193 nucleotide changes among them [23].

It is not surprising that most initial discoveries in pharmacogenetics pertained to drug-metabolizing enzymes; measurements of drugs and drug metabolites required chemical analytical methods of more or less traditional nature. Investigation of receptor variation usually requires knowledge of the receptor's DNA sequence so that deviations can be discovered by testing the receptor genes in white blood cells [24]. Thus, most studies of the pharmacogenetics of receptors or, similarly, of ion channels and of transporters, have recent origins. Many of these topics will be covered in subsequent chapters.

It is only now that we can appreciate the magnitude of genetic variation. Let us look at a few numbers: The human genome contains about 3 billion base pairs, and single-base variations (called SNPs, for single nucleotide polymorphisms) are on the average as frequent as 1/1000 bases [25]. This means that many human proteins show genetic variation. There are known, e.g., more than 100 cancer-promoting oncogenes and about three dozen cancer-suppressing genes, and their functions may be controlled by genes determining DNA repair, cell division, metabolism, immune responses, embryonic development, and cell migration [26]. Thus, the number of opportunities and the magnitude of human genetic variation explain that there is what we call pharmacogenetics.

One should not forget that many environmental factors, including drugs, produce effects by altering gene expressions. This has been known for many years, when it was realized that certain drugs, besides foods and hormones, may induce formation of drug-metabolizing enzymes. Disregarding tradition, one might consider these drug-caused alterations of gene expression as an aspect of pharmacogenetics. This will not be further discussed here.

3 PHARMACOGENETICS AND POPULATIONS

Pharmacogenetics is still largely considered a story of person-to-person differences in drug metabolism and response. A broader view becomes effective when we look at simple organisms. To appreciate the fact that pharmacogenetic variation can be a protective commodity for a population, let us consider insect resistance to insecticides [1], or bacterial resistance to antibiotics [27]. Pharmacogenetic resistance of an individual insect to the killing effect of an insecticide causes this individual to survive an exposure, so that the offspring of that insect can multiply and in the long run create the resistant strain. Bacterial resistance to antibiotics represents the same mechanism. We cannot see this dramatic effect of pharmacogenetics in people because environmental hazards are usually not so directly killing, and the human generation time is too long. The initial emphasis upon differences between individuals is changing; we interprete them increasingly as diversities which characterize different human populations [28].

I knew early on of these and some other population differences [14], but let me tell the story how the importance of such differences was driven home to me. Toronto became more and more often the home of immigrants from China. In the early 1970s, there were a few Chinese in the class of 140 medical students at the University of Toronto. My colleagues and I were studying at the time the metabolism of the then frequently used drug amobarbital, a barbiturate, and we had found a family with impaired amobarbital metabolism [29]. We therefore asked the medical students to volunteer for an amobarbital study. After we had our laboratory results, I noticed that the data from seven subjects did not fit to the rest. I suspected an error of measurement and wanted to repeat the study of these subjects. I only knew the student numbers and asked a colleague for their names; after a while, he came back—visibly shaken—that all the student numbers came from students with Chinese names. Further investigations confirmed that one of the metabolic alterations of amobarbital was on the average distinctly faster in Chinese than in Caucasian students [29].

The deficiency of debrisoquine metabolism [17] was also tested in our laboratory, and we found a different metabolic ratio between Chinese and Caucasian students [30]. These observations, together with the old G6PD and NAT2 data and some additional comparisons, firmly planted in my mind the idea that differences in drug metabolism are not only a matter of individuals but also frequently occur between the human populations. I published a review article that probably was the first exclusively concerned with interethnic differences of drug metabolism [31]. Knowledge of such differences has become very important for the pharmaceutical industry.

4 MONOGENIC AND MULTIGENIC VARIATIONS OF DRUG RESPONSES

The occurrence of the response to a drug that differs between persons can have many different causes, for instance, variability of drug metabolism as indicated above. Other potential differences may lie in drug targets or receptors, or in the transporters of drugs that operate at sites of absorption, of the blood-brain barrier, or of cellular membranes in general.

Dealing with variation of specific genes is a relatively simple affair, and so far has characterised most aspects of pharmacogenetics. However, we cannot neglect the fact that most differences between people are due to differences between many genes, in addition to environmental influences. Pharmacology became a science only after the ever-present differences between subjects were recognized and the concept formalized by introduction of the term ED_{50} [32], indicating the dose sufficient to produce a given effect in 50% of a tested population. Whether somebody belongs to the 50% needing a lower or a higher dose may depend on many factors, including drug absorption, volume of drug distribution, drug transport, blood flow, target reaction, metabolic destruction, and elimination via kidney, bile, or gut. All may contribute to a difference between two people. Every one of these factors may depend on one or more gene products. Multigenic variation is important.

Let us consider a single reaction, the metabolic alteration of a drug. The metabolism may fail because of a genetic change in the enzyme structure, as discussed above. However, it may also fail because not enough enzyme was formed, perhaps due to a failure of transcription or translation. Was there the absence of an inducer or regulator, perhaps a hormone, not formed or too fast degraded? Perhaps a genetic abnormality of the promoting region prevented the normal response to the inducer. Perhaps the drug could not reach the enzyme. Thus, most genetic differences between people are complex and many genes contribute. Because of the complexity, the causes of such differences between individuals are usually ignored. The story changes if we have to deal with a multifactorial difference between populations.

As an example, let us consider a comparison between Swedish and Chinese populations and their capacity to metabolize codeine [33]. The drug undergoes three primary metabolic reactions: glucuronidation, N-demethylation to form norcodeine, and O-demethylation to form morphine. All these reactions differ between the two populations. The slow morphine formation in Chinese reflects the known ethnic variation of CYP2D6, but no single enzyme change is known to account for the metabolic differences in glucuronidation and in nor-codeine formation. Particularly the glucuronidation curves show a normal distribution in both populations, suggesting multifactorial variation. This raises the question how

one should deal mathematically with multifactorial differences between population. As suggested in the chapter on interethnic differences in drug response, it is in such cases often better to compare the edges of two distribution curves rather than their means [34,35].

A problem with multifactorial variation is the question to what extent is it determined by heritable factors, and to what extent by environmental determinants; both are probably contributing to the variation. The answer may guide investigations of the problem: scientific inquiries may be directed to primarily look at genes or to search for environmental influences. Traditionally, twin studies were used to determine the heritability of any human variant. Since drug effects come and go, it is possible in pharmacology to avoid twins, to collect a group of people, and to give each subject a drug two or more times; this will allow a statistical comparison of inter- and intrasubject variation. The comparison can be used to calculate the genetic component contributing to any pharmacological variation [36–38].

5 ECOGENETICS AND PHARMACOGENOMICS

Observations of interindividual differences in metabolism of drugs, and therefore in different drug responses, led to the development of pharmacogenetics. However, it was not long before investigators without a particular interest in drugs noted similar differences in response to environmental toxicants. Thus, the term *ecogenetics* was coined by Brewer [39]. He asked whether geneticists with their exploding science were sufficiently concerned with humans "facing an environmental crisis of such proportions that our very existence is threatened." The term was taken up and used when concerned with genetic differences in the tolerance of food items [2], such as lactose in milk products. Calabrese [40] was much concerned with occupational diseases, and wrote a book about ecogenetics. The World Health Organization arranged in 1989 a meeting on ecogenetics, which led to a subsequent book [41]. Thus, ecogenetics is firmly established as a term and a special branch of science. The principal concepts embodied by the terms *pharmacogenetics* and *ecogenetics* are indistinguishable.*

The word *pharmacogenomics* [42,43] reflects in the first place the change of the human technical ability to investigate and to pinpoint variations in DNA, a change that encouraged geneticists to study the genome [44,45] rather than merely single genes. This is an important change: It is now realized that only a

* There is an underlying linguistic problem: The word *pharmakon* in ancient Greek refers to both drugs and poisonous substances. Therefore, the term "pharmacology" means for many medical scientists a topic dealing with both therapeutic and toxic agents. For others, pharmacology invokes thoughts of drugs as therapeutic medicines, dispensed by pharmacists. Thus, some geneticists use the term ecogenetics, though the term seemed redundant to many pharmacologists.

few parts of the human genome are the standard protein-producing genes; functions of the total genome are being explored, with the trend to compare the genomes of different species [46]. From a medical point of view, it is mainly three aspects that will make pharmacogenetics and pharmacogenomics different subjects:

1. The phenotyping methods which until recently governed most of pharmacogenetics will be more and more subservient to genotyping procedures. Phenotyping will remain important as a means to assess the medical significance of a genetic variation.
2. Looking at the genome rather than at single genes improves the chances of finding variants that promote the occurrence of common diseases, that is, diseases like blood pressure elevation, asthma, or schizophrenia. This in turn will promote the discovery of new drug targets, the genes or proteins involved in disease production.
3. Pharmacogenetics was historically most concerned with drug safety. Drug safety will remain a concern, but the main effect of pharmacogenomics promises to be an improvement of drug efficacy.

6 CONCLUSIONS

In summary, pharmacogenomics will in the long run lead to a better understanding of the interaction between drugs and gene products. The promise of pharmacogenomics is that the choice of drug to combat a disease will be determined more and more by which gene or genes contribute to the disease in a given subject; in other words, we can expect to see the development of individualized, gene-dependent drug therapy.

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