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Review

Advances in pharmacogenomics and individualized drug therapy: exciting challenges that lie ahead

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Abstract

Between the 1930s and 1990s, several dozen predominantly monogenic, high-penetrance disorders involving *pharmacogenetics* were described, fueling the crusade that gene–drug interactions are quite simple. Then, in 1990, the Human Genome Project was established; in 1995, the term *pharmacogenomics* was introduced; finally, the complexities of determining an unequivocal phenotype, as well as an unequivocal genotype, have recently become apparent. Since 1965, more than 1000 reviews on this topic have painted an overly optimistic picture—suggesting that the advent of individualized drug therapy used by the practicing physician is fast approaching. For many reasons listed here, however, we emphasize that these high expectations must be tempered. We now realize that the nucleotide sequence of the genome represents only a starting point from which we must proceed to a more difficult stage: knowledge of the function encoded and how this affects the phenotype. To achieve individualized drug therapy, a high level of accuracy and precision is required of any clinical test proposed in human patients. Finally, we suggest that metabonomics, perhaps in combination with proteomics, might complement genomics in eventually helping us to achieve individualized drug therapy.

Keywords: Pharmacogenomics; Drug therapy; Gene

Contents

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1. Introduction

By means of drug-level monitoring, the goal of clinical pharmacology for the past five decades has been to individualize the dosage of many drugs with low therapeutic indices. The best and most efficacious drug for a particular patient's condition is chosen (tailored treatment), and attempts are made to anticipate and minimize each subject's risk of experiencing an adverse drug reaction. Serious adverse drug reactions, including therapeutic failure, are common. For example, a large prospective study reported that, during a single year, more than 2 million hospitalized patients experience serious adverse drug reactions, and more than 100,000 fatalities occur as a result—ranking adverse drug reactions as the fifth leading cause of death in the United States (Lazarou et al., 1998).

Many adverse drug reactions arise because of genetic differences in drug metabolism, receptors, transporters, ion channels, and other drug targets. It would therefore be ideal if practicing physicians could test each patient—before a pharmaceutical was ever administered—to prevent most, if not all, adverse drug reactions. This form of preventive toxicity would save billions of dollars per year spent on treating adverse drug reactions and is a principal goal of pharmacogenomics. Pharmacogenomics, however, implies DNA testing. What is the present state of DNA testing in achieving individualized drug therapy? For those unfamiliar with many of the terms used in genetics and genomics, Box 1 lists definitions of all the terms used in this review.

2. Predominantly monogenic, high-penetrance pharmacogenetic disorders

Hundreds of highly successful pharmacogenetic studies showing gene–drug interactions have appeared over the past seven decades (Table 1). The hundreds of reviews on the topics of pharmacogenetics and pharmacogenomics (Weinshilboum, 2003; Evans and McLeod, 2003; Ingelman-Sundberg, 2004) have taken advantage of several of these examples in Table 1, thereby painting a very optimistic picture. Each of the apparent success stories in pharmacogenetics represents a predominantly monogenic, high-penetrance trait, in which the functional consequence of a major gene was recognized. Except for defects in three receptors (TAS2R1, RYR1, ABCC8), five transporters (ABCB1, TAP2, TAP1, SLC6A3, SLC6A4) and three channel proteins (KCNH2, CACNA1A, SCN5A), the 28 remaining of the 39 examples listed in Table 1 represent a trait described as high versus low (to nil) drug-metabolizing enzyme activity (thereby clearing any drug substrate more slowly).

The cloning and characterizations of the *CYP2D6* gene (Gonzalez et al., 1988) and the *NAT2* gene (Blum et al., 1990) were the first breakthrough examples of elucidating at the DNA level the pharmacogenetics of debrisoquine oxidation and isoniazid *N*-acetylation, respectively. This was followed by many similar studies in which additional drug-metabolizing enzyme genes—as well as some receptor, transporter and channel genes—were cloned and characterized (Table 1). It soon became clear that most, if not all, human genes have about 3 to 10 major variant alleles, and dozens or hundreds, of rare variant alleles. For example, more than 70 variant alleles have been described for the *CYP2D6* gene (Malmebo et al., 2004). The *CYP2D6* polymorphism even includes "ultra-rapid metabolizer" patients with as many as 13 copies of a functional *CYP2D6* gene in tandem; these patients are able to clear any CYP2D6 substrate extremely rapidly (Johansson et al., 1993).

It has recently been suggested (Ingelman-Sundberg, 2004) that predictive genotyping for *CYP* genes will improve clinical efficacy for all drug therapy by 15% to 25%, thereby decreasing adverse drug reactions by 10–20%. This predicted success rate, using genomics, is more reasonable than many earlier claims of 80% to 100% success rates, but we believe that even this estimate of decreasing adverse drug reactions by 10–20% may be overly optimistic, as discussed below. For example, even with the very strong single-gene high-penetrance disorder thiopurine methyltransferase (*TPMT*), a study correlating thiopurine-related adverse drug reactions with the *TPMT* genotype noted that 78% of adverse drug reactions were not associated with the *TPMT* gene polymorphism, and therefore attributable to factors other than this drug-metabolizing enzyme (van Aken et al., 2003). Any DNA test designed to detect three, or ten, DNA variant alleles for these predominantly monogenic disorders might therefore be successful in preventing some adverse drug reactions arising from these major variant alleles (Table 1). Certain vulnerable subjects including those having rare [minor allele frequency (MAF)<0.01] variant alleles, however, would probably not be discovered prior to receiving the drug and experiencing an adverse drug reaction. The clinical impact of such rare variant alleles and other predisposing factors has been underestimated in

Box 1
Definitions of terms used in this article

Term	Abbreviation	Definition
Allele		One of the different forms of the gene or DNA segment that can exist at a single locus. One allele is from the mother, the other from the father. Allelic frequencies in populations: p is the major allele; q is the variant allele(s).
Complex disease		Malady caused by one gene plus multiple environmental factors, multiple genes plus one environmental factor, or multiple genes plus multiple environmental factors (e.g., asthma, cancer, coronary heart disease, diabetes, dyslexia, hypertension, obesity, osteoporosis, stroke, stuttering).
Crossing-over		The exchange of corresponding chromosome parts by breakage and reunion. The consequence of recombination.
Drug-metabolizing enzymes	DMEs	Enzymes, numbering in the hundreds, which are capable of metabolizing pharmaceuticals.
Epigenetics		Regulatory effects on inheritance and gene expression that are not controlled by classical Mendelian genetics (e.g., DNA methylation, imprinting, RNA-mediated silencing).
Epistasis		Additive or non-additive gene interaction at a genome-wide level.
Expressivity		The variability in expression, or severity, or degree, of a trait.
Gene conversion		Gene (or part of gene) converted to the opposite allele by crossing-over.
Genocopy		When the same mutation in a particular gene leads to two different outcomes (traits) in two patients; modifier genes and environmental factors contribute to this effect.
Genotype		Genetic (DNA) sequence of each individual.
Haplotype		The relationship of variant sites (SNPs) to one another along a single chromosome.
Haplotype blocks		Segments of DNA, inherited from one generation to the next, within which variant sites are in linkage disequilibrium.
Insertions and deletions	Indels	Any number of DNA bases inserted or deleted. Comprises 5–10% of all DNA variant sites.
Linkage disequilibrium	LD	Deviation from the condition of equilibrium. Two variant sites occurring on the same chromosome more frequently than expected. Given enough time on an evolutionary time-scale, crossing-over will separate alleles at two linked loci, or two SNPs within the same large gene, that are on the same chromosome.
Mendelian		Refers to inheritance of a trait caused by two alleles of a single gene.
Minor allele frequency	MAF	Incidence of a minor allele or single-nucleotide polymorphism in population. Used more commonly today than q for a variant allele.
Non-penetrance		Failure of a trait to be evident, even though the genotype that usually causes that phenotype is present.
Non-synonymous SNP		Mutation in the DNA leading to an amino-acid change in the protein. "T164I", for example, means threonine-to-isoleucine change at residue 164 of the protein.
Penetrance		The proportion of individuals having a defined genotype who manifest a particular trait.
Pharmacogenetics		Study of the heritable response to pharmaceutical agents. Study of gene–drug interactions.
Pharmacogenomics		Study of how pharmaceutical agents interact with the total expression output of the genome, to influence biological pathways and processes. This field should help in designing new drugs.
Phenocopy		When the same trait exists in two patients as the result of different genes or environmental factors contributing to that trait.
Phenotype		Any biochemical, physiological, morphological or behavioral characteristic (trait) of an organism.

Phenotype–genotype association studies		Attempts to correlate a trait (adverse drug reaction, therapeutic failure, efficacy) with an alteration in the DNA.
Recombination		The physical process by which genes at loci on the same chromosome end up on separate chromosomes in the subsequent generation(s).
Single-nucleotide polymorphisms	SNPs	Nucleotide substitution; different base in the DNA, leading to a different base in the messenger RNA, which may or may not lead to different amino acid in the protein. *Rare* SNPs have frequencies of ≤0.01, *polymorphic* SNPs ≥ 0.01, and *common* SNPs have frequencies of 0.05 to >0.20. SNPs comprise 90–95% of DNA variant sites.

the literature. Moreover, until the function of all genes is known, the impact of pharmacogenomics itself will be limited and uncertain.

3. Polygenic traits of low and variable penetrance

More complex studies of phenotype–genotype associations began to appear during the 1990s, harbingers suggesting complications and ambiguities that clinicians might face in the future. Early reports included associations between: variations in the serotonin receptor gene (*HTR2A*) and response to clozapine (Arranz et al., 1995; Nothen et al., 1995; Burnet and Harrison, 1995; Masellis et al., 1995); a 102T>C polymorphism of the *HTR2A* gene and incidence of schizophrenia in a large multicenter study of 1210 subjects (Williams et al., 1996); and an exon 18 deletion in the α_2-macroglobulin gene (*A2M*) and risk of Alzheimer disease (Blacker et al., 1998). None of these claims was corroborated in further studies (Dow et al., 1999; Rogaeva et al., 1999; Rudrasingham et al., 1999; Moncama et al., 2002). It was suggested that the original observations might be due to regional population ethnic differences, another functional variant closely linked to the allele under study, modifier or other major genes epistatic to the allele under study, and/or another gene in linkage disequilibrium weakly associated with the trait. Also emphasized were requirements in such studies for more accurate diagnoses of disease (unequivocal phenotype), larger numbers of subjects, and reproducibility in other ethnic populations.

Several cardiovascular and asthma studies of associations have been reported, for example, between two non-synonymous mutations in the β_2-adrenergic receptor gene (*ADRB2*) and altered receptor function demonstrated in vitro (Liggett, 1997); the T164I mutation in the *ADRB2* gene and both outcome of congestive heart failure (Liggett et al., 1998) and exercise capacity in patients with heart failure (Wagoner et al., 2000); the G16R mutation in the *ADRB2* gene and response to albuterol in asthmatics (Israel et al., 2000); and the R389G mutation in the *ADRB1* gene and exercise capacity in heart failure patients (Wagoner et al., 2002). In each case, the association appears more likely to occur—by a factor of 2- to 5-fold, or even 18-fold—but certainly cannot be absolutely mandated, i.e., cannot be used with certainty by the clinician, as required for personalized medicine.

About 4% of patients receiving abacavir, an anti-human immunodeficiency virus agent, develop hypersensitivity; a recent study (Mooser et al., 2003) revealed an association between single-nucleotide polymorphisms across the *HLA* locus on chromosome 6 and hypersensitivity to abacavir. This preliminary report suggested feasibility of this approach, i.e., extended linkage disequilibrium could be detected readily, even across several haplotype blocks, thus potentially reducing the number of single-nucleotide polymorphisms for future whole-genome scans. More recent results (Anderson, 2003) including additional patients from different ethnic groups, however, showed that dozens of different positive associations occurred between *HLA* SNPs and abacavir hypersensitivity—again underscoring serious problems with successful phenotype-genotype association studies. Page et al. (2003) have suggested guidelines to address possible confounders of association before particular single-nucleotide polymorphisms or haplotypes can be regarded as "causative."

4. Difficulties in defining an unequivocal phenotype

Clear clinical descriptions are essential to define a trait unequivocally (Hall, 2003; Funalot et al., 2004). For example, the phenotype of dentatorubropallidoluysian atrophy in Japanese and European populations (Nagafuchi et al., 1994) and that of the Haw River syndrome in African-Americans (Burke et al., 1994) was sufficiently disparate to conceal the fact that these diseases arise from the same mutation. This is a good example of genocopy (Box 1).

In clinical pharmacology, phenotype denotes "any particular response seen after a drug is administered," including efficacy, therapeutic failure and toxicity. Responses to drugs can be complicated, however, by the more than two dozen problems listed in Table 2. Each factor listed can affect steps in drug absorption, distribution, metabolism and/or excretion; obviously, some effects might inhibit or cancel out other effects or, alternatively, be additive or synergistic.

It has therefore become clear just how difficult it is to identify a particular phenotype unequivocally. Individual

Table 1
Predominantly monogenic pharmacogenetic disorders that have been characterized[a]

Disorder	Major gene known to be responsible	Pivotal reference(s)
Phenylthiourea nontaster	*TAS2R1*	Snyder, 1932; Kim et al., 2003
Hypocatalasemia	*CAT*	Takahara, 1952
Atypical serum cholinesterase	*BCHE*	Kalow and Genest, 1957
Glucose-6-phosphate dehydrogenase deficiency	*G6PD*	Marks and Gross, 1959
Isoniazid slow *N*-acetylation	*NAT2*	Evans et al., 1960; Blum et al., 1990
Fish-odour syndrome trimethylaminuria	*FMO3*	Humbert et al., 1970; Hernandez et al., 2003
Debrisoquine/sparteine oxidation poor metabolizer	*CYP2D6*	Eichelbaum, 1975; Mahgoub et al., 1977; Gonzalez et al., 1988
Serum paraoxonase low activity	*PON1*	Geldmacher-von Mallinckrodt et al., 1979; Humbert et al., 1970
Thiopurine methyltransferase deficiency	*TPMT*	Weinshilboum and Sladek, 1980
Sensitivity to alcohol	*ALDH2*	Teng, 1981
S-mephenytoin oxidation deficiency	*CYP2C19*	Kupfer and Preisig, 1984; de Morais et al., 1994
Sulfotransferase deficiency	*SULT1A1, SULT1A2*	Weinshilboum, 1988
Coumarin, nicotine oxidase deficiency	*CYP2A6*	Yamano et al., 1989
P-glycoprotein transporter defect	*ABCB1*	Kioka et al., 1989
Malignant hyperthermia	*RYR1*	MacLennan et al., 1990
Quinone oxidoreductase defect	*NQO1*	Traver et al., 1992
Peptide transporter defect	*TAP2*	Powis et al., 1992
Phenytoin, warfarin oxidation defect	*CYP2C9*	de Morais et al., 1993
Debrisoquine ultra-metabolizers	*CYP2D6*1XN*	Johansson et al., 1993
Epoxide hydrolase deficiency	*EPHX1*	Hassett et al., 1994
Glutathione *S*-transferase null alleles	*GSTM1*0, GSTT1*0*	Katoh, 1994; Wiencke et al., 1995
Long-QT syndrome	*KCNH2*	Curran et al., 1995
Dihydropyrimidine dehydrogenase deficiency	*DPYD*	Meinsma et al., 1995
Dopamine transporter defect	*SLC6A3*	Sullivan et al., 1997
Chlorzoxazone hydroxylation defect	*CYP2E1*	Hu et al., 1997
Peptide transporter defect	*TAP1*	Quadri and Singal, 1998
Sulfonylurea receptor defect	*ABCC8*	Hansen et al., 1998
Calcium channel defect	*CACNA1A*	Yue et al., 1998
Serotonin transporter defect	*SLC6A4*	Smeraldi et al., 1998
Androstane glucuronosyl conjugation	*UGT2B4*	Levesque et al., 1999
Congenital long-QT syndrome	*SCN5A*	Wei et al., 1999
Caffeine 3-demethylase defect	*CYP1A2*	Nakajima et al., 1999
S-oxazepam glucuronosyl conjugation	*UGT2B7*	Strassburg et al., 2000
Paclitaxel hydroxylase deficiency	*CYP2C8*	Dai et al., 2001b
Chlorpyrifos oxidation deficiency	*CYP3A4*	Dai et al., 2001a
Nifedepine oxidation deficiency	*CYP3A5*	Lee et al., 2003
Cyclophosphamide metabolism deficiency	*CYP2B6*	Lamba et al., 2003

[a] In each case, compared with the reference allele, one or more variant alleles lead to a defective gene product, resulting in decreased metabolism, transporter or receptor activity, or channel function. The clinical consequence in most homozygous affected subjects is toxicity, due to drug accumulation with enhanced drug activity. Occasionally, decreased drug activity (therapeutic failure) ensues if the variant reflects ultra-rapid drug metabolism or if, for activity, the drug requires metabolic conversion to an active form and this conversion is decreased in the variant.

response to pharmaceuticals—just as individual response to any environmental chemical or quantitative trait mapping for any complex disease—is highly variable, forming a broad continuous gradient, similar to measurements of height, weight, or blood pressure within large populations (Nebert, 2000). A recent study employs this approach to examine quantitative trait loci influencing variation in human menopausal age (van Asselt et al., 2004). Whereas a population under study might yield a statistically significant calculated odds-ratio, there are within this

Table 2
Problems that can contribute to an equivocal phenotype[a]

Problem
Overlapping drug substrate specificity in drug-metabolizing enzymes
Overlapping drug substrate specificity in drug transporters
Overlapping drug substrate specificity in chaperones
Overlapping drug substrate specificity in receptors
Overlapping drug substrate specificity in ion channels
Overlapping drug substrate specificity in transcription factors
Metabolic pathway of drug means many enzymes, many genes
Genetic heterogeneity of drug-metabolizing enzymes
Genetic heterogeneity of drug transporters
Genetic heterogeneity of chaperones
Genetic heterogeneity of receptors
Genetic heterogeneity of ion channels
Genetic heterogeneity of second-messenger pathways
Genetic heterogeneity of transcriptional factors
Genetic heterogeneity of other drug targets not yet understood
Drug–drug interactions (induction or inhibition)
Drug–environment interactions (induction or inhibition, e.g., cigarette smoke)
Environmental factors (diet, smoking, occupational chemicals, over-the-counter natural products)
Developmental factors (age, gender, health and disease states, renal tubular excretion)

[a] Summarized from details given in Nebert et al., 2003.

population a sufficient number of outliers and subjects exhibiting differences to render problematic the likelihood of pharmacogenomics alone ever being successful in delivering individualized therapy. This means that there exist innumerable phenotype exceptions, thereby preventing the safe clinical application of such statistical results or generalizations to each individual patient. Even if we can define phenotype unequivocally, are we able to define a genotype unequivocally?

5. Difficulties in defining an unequivocal genotype

In a 1992 interview (http//www.accessexcellence.org/AB/CC/watson.html), James Watson stated that the goal of the Human Genome Project was to understand the genetic instructions for human beings. Although Watson acknowledged that completing the human genome's DNA sequence would be a big job, requiring more than 10 years, he suggested that "understanding those instructions (the DNA sequence) may consume many hundreds of years." These anticipated problems that lie ahead also apply to pharmacogenomics and individualized drug therapy.

Table 3 lists more than two dozen reasons why virtually no examples can be cited in which a single DNA variant site (genotype) is always associated with a particular trait (phenotype)—in all subjects within all human populations. In each instance listed in Table 3, there exists a reason why a genomic event or other phenomenon might override a single DNA variant site somewhere in a gene.

For example, the relatively new field of "systems biology" reflects gene–gene interactions resulting from a particular stimulus that affects a complex circuitry of pathways, ending in a response by the cell or organism. Gene–gene "sensing" or gene–gene "warfare" within the same genome has been called *molecular drive* (Dover, 2002) or *meiotic drive*. *Gene conversion* can lead to one gene "repairing," or altering the expression of, its neighboring gene. *Gene silencing* can occur by several mechanisms, including DNA hypermethylation and RNA interference. *Genomic imprinting* also results from DNA methylation. Nutrition and dietary supplementation have been shown to affect epigenetic gene regulation in humans (Waterland and Jirtle, 2003). RNA interference, caused by a class of ~22-nucleotide-long RNAs that can be further divided into small interfering RNAs (siRNAs) and microRNAs (miRNAs), plays a major role in regulation of other genes; throughout the human genome there appear to be at least 400, and perhaps as many as 2000, siRNA and miRNA genes. *Extensive transmission distortion* can lead to unequal genetic sharing among relatives. Several studies have suggested that more than 70% of all human multi-exon genes are *alternatively spliced*. *Exonic splicing enhancers* can be disabled by a synonymous single-nucleotide polymorphism, regarded by many as unimportant, compared to a non-synonymous single-nucleotide polymorphism. *Stochastic events* (random noise during transcription and other cellular processes) can also markedly affect gene expression. *Transcriptional mutagenesis* results in mutated mRNA and therefore in the protein, although the mutation does not exist in the DNA. Each of these 10 examples represents phenomena that can supersede a single DNA

Table 3
Problems that can contribute to an equivocal genotype[a]

Problem	Reference(s)
An estimated ~6 million *common* (MAF≥0.10) single-nucleotide polymorphisms	Carlson et al., 2004
An estimated ~11 million *polymorphic* (MAF≥0.01) single-nucleotide polymorphisms	Kruglyak and Nickerson, 2001
An estimated hundreds of millions *rare* (MAF≤0.01) single-nucleotide polymorphisms	Kruglyak and Nickerson, 2001; Nebert et al., 2003
Number of allelic variants in most, if not all, genes is large	Carlson et al., 2003
Number of genes contributing to any drug-response trait is large	Nebert, 2000; Nebert et al., 2003
Ethnic differences in allelic frequencies of all genes	Salisbury et al., 2003
Genocopy	Box 1
Phenocopy	Box 1
Penetrance	Box 1
Non-penetrance	Box 1
Expressivity	Box 1
Epistasis	Box 1; McGovern et al., 2003; Vieland and Huang, 2003
Epigenetics	Box 1; Sutherland and Costa, 2003
Multiple major genes plus modifier genes for most traits	King et al., 2003; Li et al., 2003
Gene–gene interactions ("systems biology")	Ideker et al., 2001; Ghaemmaghami et al., 2003
Molecular drive; meiotic drive	Dover, 2002; Pennisi, 2003
Gene conversion	Galtier, 2003; Marais, 2003; Jeffreys and May, 2004; Wall, 2004
Gene silencing	Schramke and Allshire, 2003
Genomic imprinting	Lewis et al., 2003
Extensive transmission distortion	Zollner et al., 2004
Alternative splicing (>70% of all genes)	Johnson et al., 2003
Exonic splicing enhancers	Gorlov et al., 2003
Stochastic events	Blake et al., 2003
Transcriptional mutagenesis	Doetsch, 2002
Unknown functions for conserved nongenic sequences (CNGs)	Dermitzakis et al., 2003; Inada et al., 2003; Margulies et al., 2003; Xuan et al., 2003
Genome is dynamic	Nebert, 2002
Constellations of genes that might lead to same trait (complex phenocopy)	Etzioni et al., 2003
Complexity of haplotype block formation and architecture	Crawford et al., 2004

[a] Each of these genomic phenomena can cause a particular single-nucleotide polymorphism to lose its association with a given trait.

variant site somewhere in a gene, thereby leading to the lack of an association between phenotype and genotype.

Recent comparative-genomics studies have revealed that the human and other genomes are replete with conserved nongenic sequences (CNGs) (also called conserved sequence elements, conserved noncoding sequences, and multi-species conserved sequences). The number of these CNGs—usually spanning 400–600 bases and largely unchanged over 480 million years—is more than twice the number of protein-coding genes in any vertebrate genome. CNGs are postulated to be involved in developmental and tissue- and cell-specific regulation of protein-coding genes, thereby altering phenotype. It is presently unknown how many "noncoding single-nucleotide polymorphisms" might fall within CNGs, affecting the regulation of protein-coding genes.

The genome is *dynamic* (Table 3): the entire genome, just like individual gene expression, exhibits flexibility and displays certain adaptive attributes of living, breathing organisms—somehow "sensing" and reacting when a particular gene is lost or up- or down-regulated. The genome's response to such perturbations involves expression of other genes that compensate for these aberrations. Numerous microarray studies, including total gene expression in untreated knockout mouse lines (Srivastava et al., 2002; Smith et al., 2003; Horton et al., 2003), support this concept of coordinated increases and decreases in many genes. Moreover, providing an environmental chemical as a signal to cultured cells (Puga et al., 2000) or to the intact animal (Cherkaoui-Malki et al., 2001), or administering a particular drug to a patient (Cheok et al., 2003), leads to up- and down-regulation of hundreds of genes in the genome. How many *constellations* of permutations and perturbations in gene up- and down-regulation (Etzioni et al., 2003), in response to such endogenous and exogenous stimuli, might exist, leading to the same phenotype? Consequently, increases or decreases in expression of a particular gene are likely to be independent of certain single-nucleotide polymorphisms. If there are multiple pharmacologically relevant genes being simultaneously up- and down-regulated—as a compensation for a particular gene being over- or under-expressed, or in response to an administered drug—it is likely that such "gene-gene cross-talk" would complicate, or even negate, the idea that a simple pattern of one or a few single-nucleotide polymorphisms might eventually predict accurately drug response in each patient.

Finally, there are haplotype blocks of varying sizes throughout our genome (Table 3). In general, the relationship of one DNA variant site to another, across any particular gene, is transmitted from one generation to the next. Children exhibit new haplotype patterns that differ from their parents due to the occurrence of recombination events between chromosome pairs at meiosis. This crossing-over constitutes one mechanism by which new haplotype blocks are created in each successive generation. Haplotype blocks appear to reflect regions of low recombination along a particular chromosome (Carlson et al., 2003, 2004), although simulation studies (Zhang et al., 2003; Anderson and Slatkin, 2004) suggest some blocks might appear instead as the result of genetic drift or events other than recombination hot spots. The average haplotype block appears to be 20–40 kb in length.

Instead of examining all single-nucleotide polymorphisms across the entire genome of an individual, it has been proposed (Schmith et al., 2003) that specific single-nucleotide polymorphisms might be chosen every 30 kb across the entire genome of 3 million kb, for a total of about 100,000 single-nucleotide polymorphisms. Thus, it has been suggested that a 100,000-single-nucleotide polymorphism microchip might predict accurately each individual's drug response. This intriguing proposal would then allow the practicing physician—seeing a particular pattern from a patient's 100,000-single-nucleotide polymorphism microchip—to treat patients safely with drugs and to prevent toxicity.

During the past year, an international haplotype effort, The Haplotype Map (HapMap) Project, has been initiated (Gibbs et al., 2003). The success of this multimillion-dollar effort in the human genome is based on a simple and consistent pattern of haplotype blocks across all individuals. The HapMap expects to create a tool to help researchers detect genetic contributions to many complex diseases. The theoretical advantage of the HapMap is that the total number of polymorphic single-nucleotide polymorphisms required to genotype an individual accurately might be lowered, from 11 million to perhaps 500,000.

For several reasons, we and others (Clark et al., 2003; Lonjou et al., 2003; Nebert et al., 2003) believe it unlikely that such 100,000-single-nucleotide polymorphism microchip total-genome scans, or the HapMap approach, can ever extrapolate accurately to individualized drug therapy. Haplotype blocks differ (Carlson et al., 2003, 2004; Clark et al., 2003; Crawford et al., 2004) in individuals from varying ethnic groups because the size and location of haplotype blocks arising in each generation can be affected by several events or mechanisms. Mating between ethnically diverse populations produces population admixture that results in longer linkage disequilibrium segments in second and third generations of mixed descent than in populations having a greater number of generations of mixed descent (McKeigue, 1997). This consideration is especially relevant to our discussion due to dramatically increased interbreeding worldwide during the past several decades between people of different ethnic backgrounds. Finally, the HapMap Project is concentrating on populations from only Africa, Asia and Europe. A substantial fraction of genetic variation not yet incorporated into any single-nucleotide polymorphism database includes Oceanian and Amerindian populations, which are expected to contribute as much as half the inter-continental differences in allelic frequencies (Bamshad et al., 2003). The Mouse HapMap Project is also underway (http://www.intl-pag.org/11/

11-sequenom.html), but it is unlikely that the size and locations of haplotype blocks in the mouse genome will be related to those in the human (Hellmann et al., 2003). Hence, the mechanisms underlying formation and size of haplotype blocks remain obscure; we can expect additional events and new mechanisms to be uncovered soon.

6. Exciting challenges in genomics that lie ahead

Table 4 lists the steps still needed before pharmacogenomics might bring individualized drug therapy closer to the practicing physician. First, we are far, far away from identifying every exon and every functional gene in the human genome (Oliver and Leblanc, 2004; Nelson, 2004). The same can be said for regulatory regions and CNGs, some of which can lie hundreds of kilobases from the gene transcript; a recent striking example is a *cis*-regulator of the sonic hedgehog gene (*SHH*), which sits in intron 5 of the *LMBR1* gene, at least 1 megabase from the *SHH* gene (Lettice et al., 2003).

Next, all these genes and regulatory sequences need to be completely resequenced in at least 20 subjects from each of the five major geographically separated human subgroups (Carroll, 2003; Zhivotovsky et al., 2003); the tremendous value of resequencing multiple individuals from the five major subgroups can be appreciated in studies by, for example, the Stephens (Salisbury et al., 2003) and Nickerson (Crawford et al., 2004) laboratories. This would result in discovery of all MAF>0.01 single-nucleotide polymorphisms across the entire genome of all major subgroups on the planet. Current single-nucleotide polymorphism databases categorize >40% of single-nucleotide polymorphisms either as rare (MAF<0.01), mismapped, or not polymorphic at all (Jiang et al., 2003), and our own laboratory in several ongoing SNP-discovery projects has consistently found well over half of the single-nucleotide polymorphisms that we discover do not exist in any current database.

Single-nucleotide polymorphism-typing, in at least 100 individuals of each major subgroup, should then be done to establish a "feel" for DNA variant site frequencies across all ethnic groups. From this information, perhaps tag single-nucleotide polymorphisms and haplotype blocks might help reduce the total number of single-nucleotide polymorphisms that need to be included in any phenotype-genotype association study; however, an increasing number of studies make this possibility seem doubtful. Finally, all or most of these single-nucleotide polymorphisms need to be examined in large cohorts having an unequivocal phenotype. We believe that all the steps listed in Table 4—despite all the increasingly rapid advances in technology—will take at least 10 years, if not longer. In conclusion, we can hope that Watson's prediction of "hundreds of years to understand the genome" is an exaggeration, but there are numerous reasons contained in Tables 2–4 as to why his prophecy might not be so outlandish.

Table 4
What is still needed from genomics before individualized drug treatment becomes closer to translation, i.e., used by practicing physicians

Future challenges
Identify, unequivocally, every exon of each gene in the human genome
Identify every regulatory region associated with each gene
Identify every CNG and its function in altering phenotype
Resequence across all these (genes, regulatory regions, CNGs) in at least 20 unrelated subjects from each of the five major geographically isolated subgroups on this planet [complete SNP discovery]
Consider all polymorphic (MAF≥0.01) single-nucleotide polymorphisms as potentially important
Perform SNP-typing on at least 100 unrelated subjects of each major subgroup to determine frequencies of all polymorphic (MAF≥0.01) single-nucleotide polymorphisms
Can haplotype blocks, "tag" single-nucleotide polymorphisms, etc. help?—maybe, but unlikely
Select patients or subjects with an unequivocal phenotype
Perform phenotype–genotype association studies in large cohorts (*N*=500 or more), including all five major subgroups
Establish an all-inclusive single-nucleotide polymorphism database in which a phenotype (efficacy, therapeutic failure, toxicity of a given drug at a particular dose) might be associated with genotype—including frequency of occurrence, in which ethnic group(s), and all other items listed in the last four rows of Table 2

7. Other promising research areas complementing pharmacogenomics

Genomics is making tremendous strides in understanding the etiology of complex diseases. Studying DNA markers or haplotype blocks in large, multigenerational families—some of whose members are afflicted with a complex disease—has led to successful identification of novel candidate genes. Proof that a particular gene contributes to the trait is then demonstrated by finding, in that gene, mutations that are statistically significantly associated with afflicted family members. For example, the *PAOD1* locus is associated with peripheral arterial occlusive disease (Gudmundsson et al., 2002), the *NRG1* gene plays a role in schizophrenia and has now been confirmed in two ethnically different populations (Stefansson et al., 2003), the *DYX1C1* gene plays a role in dyslexia (Taipale et al., 2003), the *PDE4D* gene contributes to stroke (Gretarsdottir et al., 2003), the *MECP2* gene is frequently defective in Rett syndrome (Miltenberger-Miltenyi and Laccone, 2003), and the *BMP2* gene plays a role in osteoporosis (Styrkarsdottir et al., 2003). In these examples, however, the mutant gene appears to determine only 15% to 35% of all cases of the trait. Accordingly, other genes must contribute—taking us back to the concept of "multiple major genes plus modifier genes," listed in Table 3.

Families containing glucocorticoid-sensitive and glucocorticoid-resistant asthmatic patients, responders and non-responders to an angiotensin-converting enzyme inhibitor, a selective serotonin reuptake inhibitor and tumor-necrosis factor-α are also currently under study by deCODE Genetics (Reykjavik, Iceland), with a claim of "an

accuracy of prediction of drug response as high as 70% to 90%." These data show that the field of genomics, using multigenerational families, is proving to be helpful in finding causative genes; however, extrapolating single-nucleotide polymorphisms in particular genes to predict accurately and unequivocally drug therapy outcome in every patient, or even the majority of patients, remains a major hurdle ahead.

As described above, many reasons exist as to why it is doubtful that genomics can supply the practicing physician with the final goal of individualized drug therapy. Are there other fields that, in the future, might complement genomics?

7.1. Transcriptomics

Transcriptomics (Kiechle and Holland-Staley, 2003) refers to the study of gene transcripts, generally analyzed by cDNA expression microarrays. Such cDNA expression studies have led to a number of exciting breakthroughs in basic science. For example, microarray analysis of certain tumors has been successful in correlating a particular microchip pattern with a patient's prognosis (McGregor, 2003). Microarrays of cDNA expression have also been used effectively as predictors of success for hormone responsiveness, hormone non-responsiveness, clinical outcomes, and tumor therapy (Domchek and Weber, 2002; Liu and Karaturi, 2004). We believe that transcriptomics is unlikely to play a major role in individualized drug therapy. The reason involves the major limitation of microarray expression: the availability of the appropriate source of sampling—blood or excreta (urine, saliva, sweat, breast milk, ejaculate, menses, feces) or tissue in which relevant cDNAs or proteins exist (dissected tumor, tissue biopsies, placenta, foreskin). It would be unethical and unreasonable to attempt to predict individual drug response outcomes from biopsies of liver, lung, kidney or brain in healthy patients.

7.2. Metabonomics

Metabonomics (also called "metabolomics") refers to the study of metabolite profiling (Plumb et al., 2002, 2003; Reo, 2002). Although this field is similarly limited by the same sampling problems as transcriptomics, there is an important difference. The metabonome represents an integrated (systems biology) response, in real time, to all endogenous plus all exogenous stimuli (drugs, chemical exposures, occupation, lifestyle, nutrition, age, gender). Metabonomics thus might provide an exquisitely sensitive means to follow an individual patient's phenotype—as a function of age, nutrition, course of disease, or therapy. Accordingly, in principle, this technique offers great promise in personalized drug therapy and medicine, but may also be subject to some of the pitfalls identified above for pharmacogenomics.

Recently, metabonomics has achieved major new advances due to novel, highly sensitive techniques for measuring urinary metabolite profiles. The analytical data in these studies are derived from electrospray mass spectrometry coupled to gas chromatography, liquid chromatography, or mass spectrometry time-of-flight. This method has proven (Plumb et al., 2002) to be more sensitive than high-resolution magic-angle spinning proton nuclear magnetic resonance (Reo, 2002). Metabolites measured include not only those from drugs, but hundreds of small-molecular-weight compounds that exist in synthetic and degradation pathways: Embden-Meyerhof glycolysis; tricarboxylic acid cycle; urea cycle; and bile acid, cholesterol, sterol, lipid, phospholipid, amino acid, sugar, fatty acid, arachidonic acid cascade, neurogenic amine, and other bioamine (synthesis and degradation) pathways.

Several animal model studies using the metabonomics approach have been reported: cortical glutamate concentrations in transgenic amyotrophic lateral sclerosis mice supplemented by creatine (Andreassen et al., 2001); response of these amyotrophic lateral sclerosis mice to Riluzole (Kaddurah-Daouk, 2003); drug-induced acute kidney and liver toxicity being studied by the Consortium for Metabolic Toxicology (Keun, 2003). Clinical studies using metabonomics have just begun (German et al., 2003). For example, patients with triple-vessel coronary heart disease of varying severity, examined by high-resolution magic-angle spinning proton nuclear magnetic resonance, exhibited greater than 90% predictability of stenosis of the major coronary vessels (Brindle et al., 2002).

Metabonomics might therefore be considered analogous to a "liver profile" test in clinical pathology, except that metabonomics includes measurement of metabolites present at much lower concentrations and, accordingly, provides several orders of magnitude greater sensitivity (femtomolar to attomolar range). Even though metabolite profiling can be performed only on easily available samples—such as blood, urine or other excreta, dissected tumor, or biopsy tissues—this method still holds promise of clinical success, and might be regarded as an extension of the present practice of clinical pharmacology (Nebert et al., 2003). A dose of a particular drug given to a particular patient can be monitored, for example, for phenotype (drug disposition, efficacy, therapeutic failure, toxicity) by means of profiling in urine not necessarily the drug metabolites, but these small-molecular-weight endogenous compounds. Such a sensitive test, revealing specific patterns of hundreds to thousands of urinary metabolites, might indicate an individual's predisposition to a toxic drug response, long before any clinical effects became evident. Metabonomics thus might aid the physician in providing each patient with personalized drug therapy—thereby avoiding toxicity and minimizing risk of adverse drug reactions.

For the past five decades, clinical pharmacologists have been using blood or urine to follow therapeutic response.

This new form of metabolite profiling would resemble what clinical pharmacology has been doing previously, but would be several orders of magnitude more sensitive in detecting subtle toxicity or other adverse drug reactions, long before these become clinically overt. Changes in an individual's metabolite profile might warrant an aggressive regimen, for example, to prevent or impede the onset of arthritis or renal disease—years before clinical symptoms appear. Therefore, because metabonomics is in many ways closest to phenotype and physiology or pathology, metabonomics might offer the best way to integrate the time- and dose-dimension, the sum of the expression output of the genome, plus all environmental and developmental factors present in each individual patient. It seems practicable that, in the more distant future, metabonomics will help genomics to revolutionize and individualize drug therapy.

7.3. Proteomics

Proteomics, the study of all proteins encoded by the genome (Campbell and Ghazal, 2004), has also been successful in certain areas of basic research. Although a recent study (Xing et al., 2004) estimated an average of 3.0 human proteins per gene, others have estimated that the true number of proteins per gene might be considerably higher. Proteomics—like transcriptomics and metabolomics—suffers from the types of source that must be sampled, e.g., blood, excreta or biopsy or tumor tissue in which relevant proteins exist. It is conceivable however that in the future proteomics investigators might identify certain protein profiles, similar to ways in which metabonomics can identify certain metabolite profiles, which might be useful for predicting adverse drug reactions long before they become overt. We expect to see during the next several years a successful complementation of genomics by both metabonomics and proteomics.

8. Conclusions

In summary, it is clear that no single approach is likely to identify all the genes responsible for all complex diseases (Dean, 2003). The same applies to identifying in all individuals the contribution of all genes and gene products responsible for a particular drug response. Also, the costs of clinical tests must be weighed against the benefits to the individual patient. Consistent with our conclusions, the U.S. Food and Drug Administration in November 2003 blocked the sale of the AmpliChip CYP450®, which Roche developed with Affymetrix, stating that a "higher level of review is required because it is of substantial importance in preventing impairment of human health, and the DNA microchip "uses sophisticated technology" (Kaiser, 2003).

New data structures and data-gathering procedures are being proposed within the PhenoFocus email chat room (http://www.phenofocus.net) and within the Human Genome Variation database (HGVbase); these interactive forums should assist in meeting the world's need for a comprehensive human phenotype-genotype database (http://hgvbse.cgb.ki.se). This approach should also help advance the cause of individualized drug therapy, as one important subset of clinical medicine.

Note added in proof

Another example of how pharmacogenomics has been successful was recently reported (Lynch et al., 2004; Paez et al., 2004). Less than 10% of Caucasians, but about 25% of Japanese, with non-small-cell lung cancer respond favorably to gefitinib (Iressa®)—if they have particular mutations in the tyrosine kinase domain of the epidermal growth factor receptor gene (*EGFR*) of their tumor. The findings suggest there is no need to try this anti-cancer drug in patients not having this tumor *EGFR* genotype. There are patients without any apparent mutation who do respond, and patients with mutations who do not respond, to gefitinib, however, further underscoring the problems with phenotype-genotype association studies and individualized drug therapy for all patients—which have been presented in this overview.

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