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Pharmacogenomics

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INTRODUCTION

Previous chapters have described drugs in the context of their molecular targets and their metabolism. Although modern pharmacologic agents can be used successfully to treat or control diseases that range from hypertension to infection with human immunodeficiency virus (HIV), there are large individual variations in response to drug therapy. These variations can range from potentially life-threatening adverse drug reactions to equally serious lack of therapeutic efficacy. Many factors can influence the drug response phenotype, including age, gender, and underlying disease, but genetic variation also plays an important role. Interindividual differences in the genes that encode drug targets, drug transporters, or enzymes that catalyze drug metabolism can affect profoundly the success or failure of pharmacotherapy.

Pharmacogenetics is the study of the role of inheritance in variation in drug response. The convergence of recent advances in genomic science and equally striking advances in molecular pharmacology has resulted in the evolution of pharmacogenetics into pharmacogenomics—terms that are often used interchangeably. The promise of pharmacogenetics-pharmacogenomics is the possibility that knowledge of a patient's DNA sequence could be used to enhance pharmacotherapy, maximizing drug efficacy by targeting drugs only to those patients who are most likely to benefit while, at the same time, reducing the incidence of adverse drug reactions.

This chapter describes the principles of pharmacogenetics and pharmacogenomics as well as recent developments in this discipline. Examples are cited in which knowledge of pharmacogenetics-pharmacogenomics can help individualize drug therapy.

Case

Robert H, a 66-year-old man, is shoveling snow one wintry morning in Minnesota when he slips and falls on a patch of ice. He immediately feels pain in his left hip and is unable to stand. He is brought to the hospital, where x-rays reveal that he has fractured his hip. He undergoes surgery the next day and is discharged to a rehabilitation hospital 3 days later. After less than 24 hours at the rehabilitation hospital, Mr. H develops the sudden onset of pleuritic chest pain. He is brought to the Emergency Department, where a CT scan with intravenous contrast reveals a pulmonary embolus. He is treated with heparin and is anticoagulated with warfarin at a starting dose of 5 mg each day, with a target international normalized ratio (INR) of 2.0–3.0. Mr. H is discharged back to his rehabilitation hospital and referred to his local physician. When the INR is subsequently measured, it is 6.2, a value associated with an increased risk of hemorrhage. He is taking no other medication that might interfere with plasma levels of warfarin. The physician advises Mr. H to stop taking warfarin for 2 days. After multi-

ple attempts at adjusting his dose of warfarin, Mr. H eventually reaches a stable INR of 2.5 when taking 1 mg of warfarin each day.

QUESTIONS

1. What additional laboratory information could assist in anticoagulating this patient?
2. Would that information have helped in the selection of Mr. H's initial warfarin dose?
3. What molecular mechanisms could be responsible for the apparent sensitivity of this patient to warfarin?

PHYSIOLOGY

Three types of interindividual genetic variation can influence pharmacotherapy: variation in proteins involved in drug metabolism or transport (pharmacokinetic variation); variation in drug targets or pathways associated with those targets (pharmacodynamic variation); and genetic variation associated with idiosyncratic drug effects.

GENOMIC VARIATION AND PHARMACOGENOMICS

The human genome contains approximately three billion nucleotides. Current estimates are that the genome contains between 25,000 and 40,000 genes that, through alternative splicing and posttranslational modification, may encode 100,000 or more proteins. Any two people differ on average at about one nucleotide in every 1,000 in their genome, totaling an average interindividual difference of 3 million base pairs throughout the genome. The majority of these differences are so-called **single nucleotide polymorphisms** or **SNPs** (pronounced "snips"), in which one nucleotide is exchanged for another at a given position. SNPs and other differences in DNA sequence can occur anywhere in the genome, in both coding regions and noncoding regions. If a SNP changes the encoded amino acid, it is called a nonsynonymous coding SNP (cSNP). The remaining differences in DNA sequence involve insertions, deletions, duplications, and reshufflings, sometimes of just one or a few nucleotides but occasionally of whole genes or larger DNA segments that include many genes. Functionally significant DNA sequence differences tend to fall within genes, either within their coding sequences or in the promoters, enhancers, splice sites, or other sequences that control gene transcription or mRNA stability. Taken together, these differences constitute each person's genetic individuality. Some of that individuality affects the way in which each person will respond to drug treatment.

PHARMACOLOGY

The concept that inheritance might be an important determinant of individual variation in drug response emerged half a century ago. It originally grew out of clinical observations of striking differences among patients in their response to "standard" doses of a drug. Those observations, in addition to twin and family studies that showed inherited variations in plasma drug concentrations and other pharmacokinetic parameters, led to the birth of pharmacogenetics. Many of those original examples of pharmacogenetic variation, and many of the most striking examples even today, involve *pharmacokinetics*—factors that influence the concentration of drug reaching its target(s). However, examples of pharmacogenetic variation in the drug target, so-called *pharmacodynamic factors,* are also being reported with increasing frequency.

VARIATION IN ENZYMES OF DRUG METABOLISM: PHARMACOKINETICS

Inherited variation in enzymes that catalyze drug metabolism is the most common factor responsible for pharmacogenetic variation in response to medications. The enzymes involved in drug metabolism are discussed in Chapter 4, Drug Metabolism. There are two broad categories of drug-metabolizing enzymes: those that catalyze phase I reactions (functionalization reactions that typically involve oxidation or reduction) and those that catalyze phase II reactions (typically, conjugation reactions that add groups, such as glucuronic acid, that enhance drug solubility and thus drug excretion). Phase I and phase II reactions do not necessarily occur in that order, and metabolic intermediates resulting from both types of reactions may be pharmacologically active. In fact, some medications are administered as inactive prodrugs that must undergo phase I and/or phase II metabolism before they can exert their pharmacologic effect.

Genetic polymorphisms are common in enzymes that catalyze drug metabolism, and clinically significant polymorphisms have been found in nearly all of the major enzymes involved in both phase I and phase II reactions (Table 52-1). Two "classic" examples are provided by the inherited variations in the enzymatic hydrolysis of the short-acting muscle relaxant succinylcholine by the enzyme butyrylcholinesterase (BChE) and the enzymatic acetylation of drugs such as the antituberculosis drug isoniazid (see Chapter 33, Pharmacology of Bacterial Infections: Cell Wall Synthesis). Patients with variations in BChE have a decreased rate of metabolism of acetylcholine and its analogues, resulting in prolonged paralysis after drug exposure. A genetically polymorphic phase II enzyme, N-acetyltransferase 2 (NAT2), catalyzes the acetylation of isoniazid. Patients treated with isoniazid can be classified as either "slow acetylators" who metabolize isoniazid slowly and have high blood drug levels, or "fast acetylators" who metabolize isoniazid rapidly and have low blood drug levels. Family studies have shown that the rate of isoniazid biotransformation is inherited. The slow-acetylator phenotype is associated with drug toxicities that result from excessive drug accumulation; examples include hydralazine- and procainamide-induced lupus and isoniazid-induced neurotoxicity. Although the antihypertensive agent hydralazine is rarely used today in the treatment of hypertension, this drug has recently re-emerged as one of the two active components in BiDil, a combination drug

TABLE 52-1 Examples of Genetic Polymorphisms and Drug Metabolism

ENZYME	AFFECTED DRUG, CLASS, OR COMPOUND
Phase I (Oxidation/Reduction) Enzyme	
CYP1A2	Acetaminophen, caffeine, propranolol
CYP1B1	Estrogens
CYP2A6	Halothane, nicotine
CYP2B6	Cyclophosphamide
CYP2C8	Paclitaxel, retinoic acid
CYP2C9	Nonsteroidal anti-inflammatory drugs, phenytoin, warfarin
CYP2C19	Omeprazole, phenytoin, propranolol
CYP2D6	Antidepressants, β-adrenergic antagonists, codeine, debrisoquine, dextromethorphan
CYP2E1	Acetaminophen, ethanol
CYP3A5	Calcium channel blockers, cyclosporine, dapsone, etoposide, lidocaine, lovastatin, macrolides, midazolam, quinidine, steroids, tacrolimus, tamoxifen
Phase II (Conjugation) Enzyme	
N-Acetyltransferase 1	Sulfamethoxazole
N-Acetyltransferase 2	Dapsone, hydralazine, isoniazid, procainamide, sulfonamides
Sulfotransferases (SULTs)	Acetaminophen, dopamine, epinephrine, estrogens
Catechol-O-methyltransferase	Catecholamines, levodopa, methyldopa
Histamine N-methyltransferase	Histamine
Thiopurine S-methyltransferase	Azathioprine, mercaptopurine, thioguanine
UDP-glucuronosyltransferases	Androgens, ibuprofen, irinotecan, morphine, naproxen

approved for the treatment of patients with symptomatic heart failure. It is of interest that the U. S. Food and Drug Administration (FDA) has approved BiDil for use only in patients of African ancestry, presumably because of an ethnically dependent genetic difference in response to this drug.

Early examples of pharmacogenetics, such as those represented by BChE and NAT2, served as a stimulus to search for additional examples. Most of the second-generation examples continue to be associated with pharmacokinetics and continue to be recognized from clinical observations—often from adverse drug responses. They have been studied most often either by administering a ''probe drug'' to a group of subjects and measuring plasma or urinary drug and/or metabolite concentrations, or by directly assaying a drug-metabolizing enzyme in an easily accessible tissue such as the red blood cell (e.g., a series of methyltransferase enzymes). Two prototypic examples that have become pharmacogenetic ''icons'' are the **cytochrome P450 2D6 (CYP2D6)** and **thiopurine S-methyltransferase (TPMT)** genetic polymorphisms. Because of the clinical implications of these polymorphisms, the FDA in its 2003 ''Guidance on Pharmacogenomic Data'' cited CYP2D6 and TPMT as examples of valid pharmacogenomic biomarkers.

CYP2D6 is a member of the cytochrome P450 (CYP) family of microsomal, phase I drug-metabolizing enzymes. CYP2D6 contributes to the metabolism of a large number of medications, including antidepressants, antiarrhythmics, and analgesics. The CYP2D6 polymorphism was originally described by two different laboratories studying two different probe drugs, the antihypertensive **debrisoquine** and the oxytotic agent **sparteine**. The frequency distribution of the debrisoquine urinary metabolic ratio, the ratio of the parent drug to its oxidized metabolite, is shown in Figure 52-1A for a Northern European population. Shown at the far right of the figure is a group of ''poor metabolizers'' of debrisoquine, subjects homozygous for recessive alleles (genes) coding for enzymes with decreased activity; shown in the middle is the large group of ''extensive metabolizers,'' subjects heterozygous or homozygous for the ''wild type'' allele; and shown at the far left is a small subset of ''ultrarapid metabolizers,'' some of whom have multiple copies of the CYP2D6 gene.

Several molecular genetic mechanisms are responsible for variation in CYP2D6 enzyme activity, including nonsynonymous cSNPs, gene deletion, and gene duplication; some ultrarapid metabolizers can have up to 13 copies of the gene. It has been estimated that 5% to 10% of Caucasians are CYP2D6 poor metabolizers. Among East Asians, in contrast, the poor-metabolizer phenotype is present at a frequency of just 1% to 2%. The ultrarapid metabolizer phenotype, rare in most Caucasian populations, has a frequency of 3% in Spaniards and up to 13% in Ethiopians. These ethnic differences have potentially important medical implications, because CYP2D6 metabolizes many commonly prescribed medications, including the β-adrenergic blocker **metoprolol**, the neuroleptic **haloperidol**, the opioids **codeine** and **dextromethorphan**, and the antidepressants **fluoxetine**, **imipramine**, and **desipramine**, among many others (Table 52-1). Therefore, poor metabolizers for CYP2D6 can potentially experience an adverse drug effect when treated with standard doses of agents such as metoprolol that are inactivated by CYP2D6, whereas codeine is relatively ineffective in poor metabolizers because it requires CYP2D6-catalyzed metabolism to form the more potent opioid morphine. Conversely, ultrarapid metabolizers may require unu-

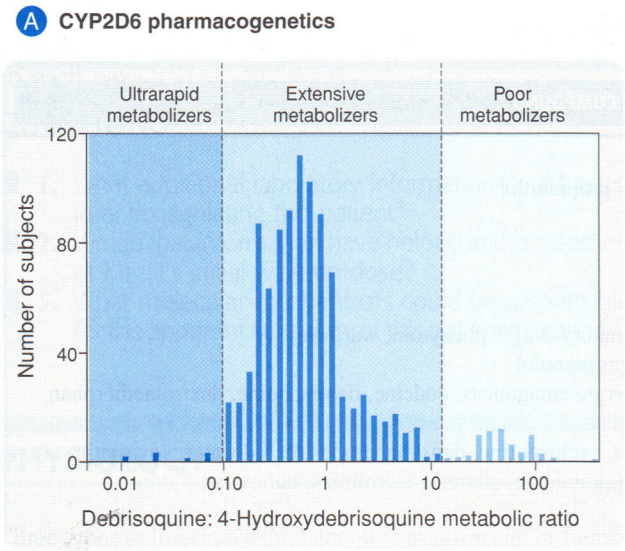

Figure 52-1. CYP2D6 pharmacogenetics. A. Frequency distribution of the metabolic ratio for the cytochrome P450 2D6 (CYP2D6)-catalyzed metabolism of debrisoquine to form its 4-hydroxy metabolite. Data for 1,011 Swedish subjects are plotted as the ratio of metabolites in the urine. Most subjects metabolize debrisoquine extensively, while some subjects metabolize the compound ultrarapidly and others metabolize the compound poorly. **B.** The AmpliChip CYP450 array can be used to determine variant genotypes for cytochrome P450 genes that influence drug metabolism.

sually high doses of drugs that are inactivated by CYP2D6, but those same subjects can be "overdosed" with codeine, suffering respiratory depression or even respiratory arrest in response to "standard" doses. In the past, an individual's genotype for CYP2D6 and many other genes encoding drug-metabolizing enzymes was inferred from phenotype, e.g., the urinary metabolic ratio that can be measured by assaying the urinary excretion of a specific metabolite after the administration of a probe drug (Fig. 52-1A). As discussed below, genotype assignment is now increasingly dependent on DNA-based tests performed with devices such as the "chip" shown in Figure 52-1B.

Thiopurine S-methyltransferase (TPMT) represents another example of an important and clinically relevant genetic polymorphism for drug metabolism. This example has also served as an important pharmacogenetic model system. TPMT catalyzes the S-methylation of thiopurine drugs such as **6-mercaptopurine** and **azathioprine** (Chapter 37, Pharmacology of Cancer: Genome Synthesis, Stability, and Maintenance). Among other indications, these cytotoxic and immunosuppressive agents are used to treat acute lymphoblastic leukemia of childhood and inflammatory bowel disease. Although thiopurines are useful drugs, they have a narrow therapeutic index, i.e., the difference between the toxic and therapeutic dose is small, with occasional patients suffering from life-threatening thiopurine-induced myelosuppression.

In Caucasians, the most common variant allele for TPMT is *TPMT*3A*; the gene frequency is approximately 5%, so 1 in 300 subjects carries two copies of the TPMT*3A allele. TPMT*3A is predominantly responsible for the trimodal frequency distribution of the level of red blood cell TPMT activity shown in Figure 52-2. TPMT*3A has two nonsynonymous cSNPs, one in exon 7 and another in exon 10 (Fig. 52-2). The presence of TPMT*3A results in a striking decrease in tissue levels of TPMT protein. Mechanisms responsible for the observed decrease in TPMT*3A protein level

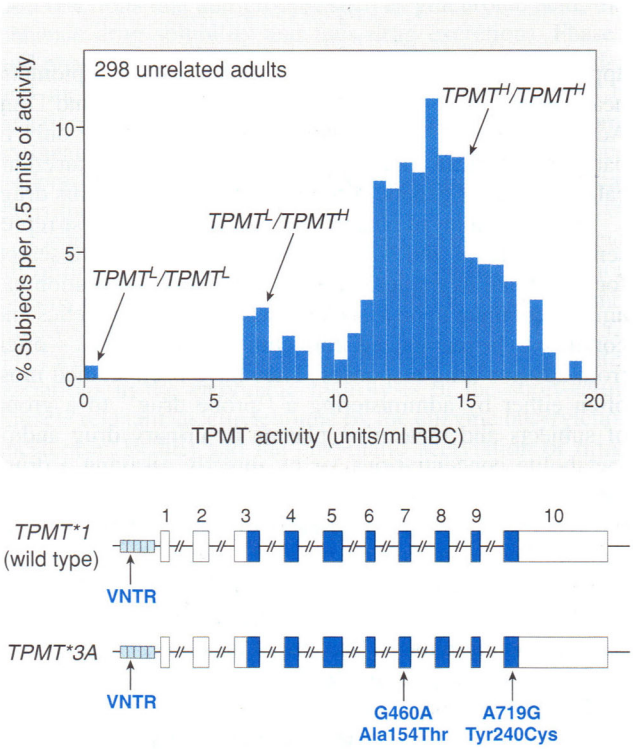

Figure 52-2. TPMT pharmacogenetics. Frequency distribution of red blood cell (RBC) thiopurine S-methyltransferase (TPMT) activity for 298 unrelated Caucasian subjects. *TPMT*^{L} indicates an allele or alleles for the trait of low activity, while *TPMT*^{H} refers to the "wild type" (*TMPT*1*) allele for high activity. The observed trimodal frequency distribution for RBC TPMT activity is due mainly to the effect of *TPMT*3A*, the most common variant allele for low activity in a Caucasian population. *TMPT*1* and *TPMT*3A* differ by two nonsynonymous single nucleotide polymorphisms (SNPs), one in exon 7 and one in exon 10.

include both accelerated TPMT*3A degradation and intracellular TPMT*3A aggregation, probably as a result of protein misfolding. As a result, drugs such as 6-MP are poorly metabolized and may reach toxic levels. *Subjects homozygous for TPMT*3A are at greatly increased risk for life-threatening myelosuppression when treated with standard doses of thiopurine drugs.* These patients have to be treated with approximately one-tenth to one-fifteenth the standard dose. There are striking ethnic differences in the frequency of variant alleles for TPMT. For example, TPMT*3A is rarely observed in East Asian populations, while *TPMT*3C*, which has only the exon 10 SNP, is the most common variant allele in those populations.

Because of its clinical significance, TPMT was the first example selected by the FDA for public hearings on the inclusion of pharmacogenetic information in drug labeling. For the same reason, clinical testing for TPMT genetic polymorphisms is widely available. The phenomenon of marked changes in the level of a protein as a result of the alteration of only one or two amino acids in the protein has been observed repeatedly for many other genes of pharmacogenetic significance and is a common explanation for the functional effects of nonsynonymous cSNPs.

The BChE, NAT2, CYP2D6, and TPMT genetic polymorphisms all behave as monogenic (single-gene) Mendelian traits, as do many other early examples from pharmacogenetics. However, pharmacogenetics-pharmacogenomics has now moved beyond monogenic pharmacokinetic traits, and the focus increasingly involves functionally and clinically significant variation in drug targets as well as drug-metabolizing enzymes. Variation can also involve multiple genes that influence both pharmacokinetics and pharmacodynamics.

VARIATION IN DRUG TARGETS: PHARMACODYNAMICS

Drugs generally exert their effects by interacting with specific target proteins. Therefore, genetic variations in these target proteins, or in signaling pathways downstream from the target proteins, can influence the outcome of pharmacotherapy (Table 52-2). Furthermore, variation in drug targets can occur either as a result of variation in germ-line DNA or, in the case of cancer, through variation in somatic DNA present in the tumor. One example of genetic variation for a drug target in germ-line DNA involves a class of drugs that is used to treat asthma. As noted in Chapter 46, Integrative Inflammation Pharmacology: Asthma, the antiasthma medication **zileuton** decreases airway inflammation by inhibiting the enzyme **5-lipoxygenase,** an enzyme encoded by the gene ***ALOX5***. Variations in 5-lipoxygenase illustrate the point that variation in many areas of a gene can affect protein function. The functional significance of nonsynonymous cSNPs—and their ability to alter the amount of protein expressed—was highlighted in the previous section on TPMT pharmacogenetics. In addition, however, polymorphisms in regulatory regions, such as the gene promoter, can influence transcription and thereby alter protein expression. The promoter of the *ALOX5* gene displays variation in the number of tandem repeats of the sequence GGGCGG. These repeat sequences bind the transcription factor complex Sp1, which up-regulates *ALOX5* transcription.

The most common *ALOX5* allele contains five repeats and is present in about 77% of *ALOX5* genes. As a result, approximately 94% of the population has at least one copy of the five-repeat allele. The most common variant alleles contain four and three repeats and are present at frequencies of about 17% and 4%, respectively. Because of increased Sp1 binding, people who carry the five-repeat allele are thought to express more 5-lipoxygenase than those who lack it. Interestingly, there seems to be no relationship between the presence or absence of the five-repeat allele and the severity of asthma in the population; i.e., this *ALOX5* promoter polymorphism does not seem to affect the disease process itself. However, in trials of a 5-lipoxygenase inhibitor related to zileuton, only subjects who had at least one copy of the five-repeat allele responded to the drug. This result suggests that zileuton-like compounds are unlikely to help the 6% of the population who lack the five-repeat allele, and that identifying this subgroup would allow the use of alternative, more effective, medications. *This example also illustrates an important principle, that a polymorphism need not cause a disease to influence the treatment of that disease.*

An example of genetic variation in a drug target in somatic (tumor) DNA involves gain-of-function mutations in the gene encoding the **epidermal growth factor receptor (EGFR)** (also known as *HER1* or *ErbB1*) in patients with nonsmall cell lung cancer (NSCLC). Two groups reported in 2004 that, in patients with NSCLC, response to the EGFR inhibitor **gefitinib** was influenced strongly by these somatic DNA mutations, i.e., subjects with sequence variation in the portion of the gene encoding the ATP binding site of this receptor tyrosine kinase responded more favorably to gefitinib therapy than did patients without such mutations.

TABLE 52-2 Examples of Genetic Polymorphisms and Drug Targets

PROTEIN	AFFECTED DRUG CLASS (EXAMPLE)
5-Lipoxygenase	Zileuton
Angiotensin-converting enzyme (ACE)	ACE inhibitors (lisinopril)
Apolipoprotein E	Statins (pravastatin)
β_2-Adrenergic receptor	β-Adrenergic agonists (albuterol)
Epidermal growth factor receptor	Gefitinib
Sulfonylurea receptor	Tolbutamide
Vitamin K epoxide reductase complex 1	Warfarin

EGFR is often overexpressed in these tumors, and several drugs targeting this receptor have been tested clinically. It was already known that NSCLC patients of East Asian origin responded more favorably to gefitinib therapy than Caucasian patients did, and one of the two original studies reported that somatic mutations in *EGFR* occurred in 15 of 58 randomly selected tumors obtained from patients in Japan but in only 1 of 61 from the United States—illustrating, once again, striking ethnic differences in pharmacogenetic effects. The example provided by gefitinib may represent the future of oncology, where both somatic and germ-line mutations/polymorphisms may be considered prior to initiating a therapeutic program. This example and the *ALOX5* example also demonstrate that pharmacodynamic-pharmacogenetic variation (i.e., variation in genes encoding drug targets) can be just as important, if not more important, than the pharmacokinetic-pharmacogenetic variation represented by CYP2D6 and TPMT. Table 52-2 lists several polymorphisms in genes encoding drug target proteins that have been associated with variation in drug response.

PATHWAY-BASED PHARMACOGENETICS-PHARMACOGENOMICS

The preceding examples, *CYP2D6*, *TPMT*, *ALOX5*, and *EGFR*, all involve clinically significant pharmacogenetic variation as a result of sequence variation in a single gene, i.e., monogenic inheritance. Figure 52-3 illustrates this pharmacokinetic-pharmacodynamic pharmacogenomic dichotomy, using the four major examples cited in this chapter. However, it is also possible for multiple genes encoding proteins that influence both pharmacokinetics and pharmacodynamics to alter the drug response phenotype.

One good example is provided by the anticoagulant warfarin. Warfarin (see Chapter 22, Pharmacology of Hemostasis and Thrombosis) is one of the most widely prescribed oral anticoagulants in both North America and Europe. However, in spite of the existence of a laboratory test that is used universally to follow warfarin's effect on coagulation (INR), serious adverse reactions—involving both hemorrhage and undesired thrombosis—continue to complicate warfarin therapy. These complications are illustrated by the case of Mr. H at the beginning of this chapter: after a ''standard'' dose of warfarin, his INR was elevated to 6.2, a level associated with increased risk for hemorrhage.

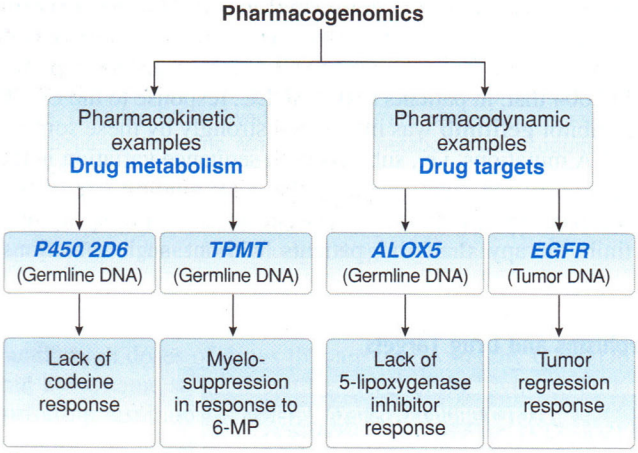

Figure 52-3. Pharmacokinetic and pharmacodynamic pharmacogenomics. The figure depicts the major pharmacokinetic (drug metabolism) and pharmacodynamic (drug target) examples described in this chapter. Shown are the affected gene *(in italics)*, whether germ-line or somatic (e.g., tumor) DNA is involved, and the clinical response observed in the presence of the variant allele(s). *P450 2D6*, cytochrome P450 2D6 gene; *TPMT*, thiopurine S-methyltransferase gene; *ALOX5*, 5-lipoxygenase gene; *EGFR*, epidermal growth factor receptor gene; 6-MP, 6-mercaptopurine.

Why might that have occurred? First, we need to remember that warfarin is a racemic mixture. S-warfarin is three to five times more potent than R-warfarin, and S-warfarin is metabolized predominantly by the cytochrome P450 isoform **CYP2C9**. CYP2C9 is a highly polymorphic gene, and the variant alleles *CYP2C9*2* (Arg144Cys) and *CYP2C9*3* (Ile358Leu) are associated with only 12% and 5%, respectively, of the level of enzyme activity observed with the wild-type allele (*CYP2C9*1*). Patients who carry these variant alleles require decreased doses of warfarin to achieve an anticoagulant effect, and these same subjects have increased risk for hemorrhage during warfarin therapy. However, this pharmacokinetic-pharmacogenetic variation fails to explain most of the variance in the therapeutic warfarin dose in patients who are anticoagulated with this powerful, but potentially dangerous, drug.

The molecular target for warfarin was not identified until 2004. The gene encoding that target, **vitamin K epoxide reductase complex 1, *VKORC1***, was also cloned that year. When the *VKORC1* gene was sequenced in a number of patients, although no nonsynonymous cSNPs were found, a series of haplotypes (combinations of SNPs on a single chromosome) was observed that were associated with warfarin dose requirement. In one study, patients with *VKOCR1* haplotypes that were associated with a low dose requirement had an average warfarin maintenance dose approximately half of that required by subjects with haplotypes associated with a high dose requirement. Several subsequent studies have confirmed that *VKORC1* haplotype is associated with approximately 25% to 30% of the variance in warfarin maintenance dose, while 5% to 15% can be explained by *CYP2C9* genotype. The roles of CYP2C9 and VKORC1 in warfarin pharmacokinetics and pharmacodynamics are shown schematically in Figure 52-4. Because the genes encoding both of these proteins contribute to variation in drug response, genotyping for *CYP2C9* as well as haplotyping for *VKORC1* could represent a useful strategy for the determination of an initial warfarin dose for Mr. H.

Warfarin provides a striking example of a situation in which pharmacokinetic-pharmacogenetic data proved inadequate for clinical translation because those data explained too little of the variation in therapeutic drug dose. However, when *CYP2C9* polymorphisms and *VKORC1* haplotypes were both determined, it became possible to assess genetic variation both in drug metabolism and in the drug target, and to move beyond the monogenic pharmacogenetics represented by NAT2, CYP2D6, and TPMT. Therefore, warfarin represents, probably in a simplified form, the type of polygenic, pathway-based pharmacogenetic-pharmacogenomic model that may become increasingly common in the future.

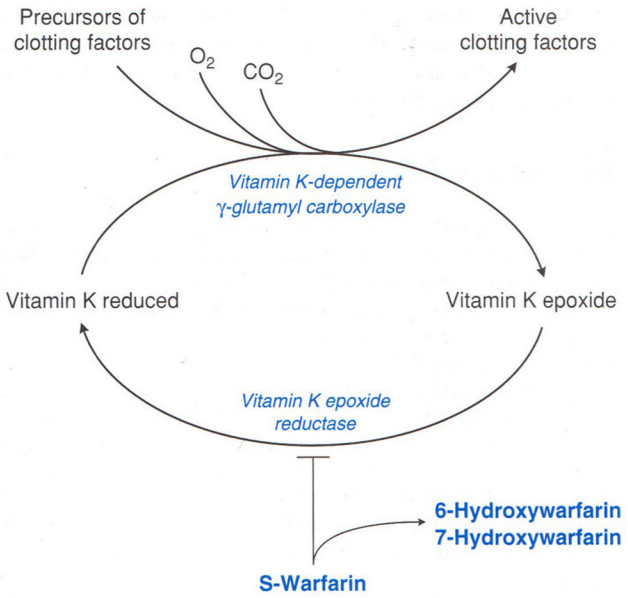

Figure 52-4. Warfarin pharmacokinetics and pharmacodynamics. Vitamin K is a required cofactor for the posttranslational γ-carboxylation of glutamate residues in certain clotting factor precursors (see Chapter 22). Vitamin K is oxidized to the inactive epoxide as a consequence of the carboxylation reaction. The enzyme vitamin K epoxide reductase (VKORC1) converts the inactive epoxide into the active, reduced form of vitamin K. Warfarin acts as an anticoagulant by inhibiting VKORC1 and thereby preventing the regeneration of reduced vitamin K. S-warfarin is metabolized to 6-hydroxywarfarin and 7-hydroxywarfarin by cytochrome P450 2C9.

IDIOSYNCRATIC DRUG REACTIONS

An additional way in which genetic variation might influence pharmacotherapy involves idiosyncratic drug reactions. These effects differ from the other examples described in this chapter in that they are not known to be caused by differences in either drug metabolism or drug targets. Instead, idiosyncratic effects seem to result from interactions between the medication and a unique aspect of the physiology of the individual patient. A ''classic'' example illustrating this least predictable effect of interindividual genetic variation is represented by idiosyncratic drug reactions associated with functional deficiency of the enzyme **glucose 6-phosphate dehydrogenase** (G6PD; see Chapter 35, Pharmacology of Parasitic Infections). This enzyme is involved in protecting red blood cells from oxidative injury. A number of polymorphisms result in this condition. The most common involves a cSNP that causes an amino acid substitution, resulting in a 90% to 95% reduction of G6PD enzyme function. That allele, A^-, is present in 10% to 20% of Africans and is thought to provide protection against malaria. A different G6PD-inactivating polymorphism is found at lower frequency among subjects of Mediterranean, Middle Eastern, Indian, and Southeast Asian descent, and a third polymorphism is also present in Southeast Asia. A number of medications cause oxidative stress on red blood cells as an effect unrelated to their intended targets or their metabolic clearance. These drugs include sulfonamides, antimalarials, and the analgesic agents acetaminophen and ibuprofen, among others. Individuals with G6PD deficiency who are exposed to these medications may develop acute and, at times, severe hemolytic anemia.

By definition, idiosyncratic effects are difficult or impossible to predict. However, information emerging from genomic, proteomic, and metabolomic research may prove useful in the future in the development of pharmacogenomic screens for unanticipated drug interactions. At present, unfortunately, idiosyncratic effects cannot be predicted.

MODERN PHARMACOGENETICS-PHARMACOGENOMICS

Completion of the Human Genome Project and the ongoing refinement of the HapMap project point the way to future developments in pharmacogenetics and pharmacogenomics in this ''post-genomic'' era. Application of modern genomic assay techniques, when combined with an increasing focus on pharmacokinetic pathways—pathways that encompass genes encoding all of the drug-metabolizing enzymes and transporters that could influence the final concentration of drug reaching the target—together with pharmacodynamic pathways that include the drug target and signaling pathways downstream from that target, may represent the future for this aspect of ''individualized medicine.'' To attain the goal of truly personalized drug therapy, and to translate genomic knowledge fully into clinical practice, will require the clinical application of high-throughput genotyping technologies. Numerous platforms for genotyping have been developed and refined, and new ones are being developed. As an example, the CYP450 gene chip shown in Figure 52-1B has already been introduced into clinical practice. To apply genotype information to select responsive patients, and then to treat these patients pharmacologically on the basis of genotype, will require a comprehensive knowledge of genotype-phenotype correlations.

However, to achieve truly individualized drug therapy, we need not only to understand the science underlying pharmacogenetics and pharmacogenomics and to develop state-of-the-art technologies to detect and assay DNA sequence data, but also to translate that knowledge into the clinic. That translation process will require active involvement of the FDA and the pharmaceutical industry, which develops virtually all new drugs. In 2003 the FDA issued a Draft Guidance with regard to pharmacogenomic data, and this draft was approved in 2005. The FDA also initiated a series of public hearings with regard to the incorporation of pharmacogenomic data into drug labeling. These hearings began with thiopurine drugs and TPMT, and were followed by hearings on a genetic polymorphism in *UGT1A1*, a gene encoding a phase II enzyme involved in the biotransformation of the antineoplastic agent irinotecan. Public hearings have recently been held on *CYP2C9*, *VKORC1,* and warfarin.

The attention given to pharmacogenetics-pharmacogenomics by the FDA is having an impact on the pharmaceutical industry, especially within the context of the unfortunate series of events that resulted in the withdrawal of the COX-2 inhibitor rofecoxib (Vioxx) from the market for reasons of safety. It is unclear whether pharmacogenetics played a role in the Vioxx-induced cardiovascular disease that led to the withdrawal of that drug. However, pharmacogenetics

almost certainly could contribute to postmarketing surveillance, not only to help avoid adverse reactions, but also to help "rescue" drugs that might be of benefit to groups of patients selected on the basis of genetic variation in drug response. The latter situation was recently highlighted when a polymorphism in the β_1 adrenoceptor was shown to influence response to the β_1-adrenergic antagonist bucindolol—both in vitro and in patients with heart failure. This β-antagonist had initially failed in a clinical trial that did not include genotyping, probably because only patients with the wild type β_1-adrenoceptor genotype had the desired clinical response.

Conclusion and Future Directions

Pharmacogenetics and pharmacogenomics involve the study of ways in which gene sequence variation affects the response of individual patients to medications. The goal of pharmacogenetics and pharmacogenomics is to maximize efficacy and minimize toxicity, based on knowledge of an individual's genetic composition. Although many factors other than inheritance influence differences among patients in their response to drugs, the past half-century has demonstrated that genetics is an important factor responsible for variation in the occurrence of adverse drug reactions or the failure of individual patients to achieve the desired therapeutic response. Pharmacogenetics has evolved during that half-century from classical examples, such as CYP2D6 and TPMT, to include more complex situations such as that represented by warfarin pharmacogenetics, involving both pharmacokinetic and pharmacodynamic pharmacogenetic variation. This area of genomic medical science also presents unique challenges in its translation into the clinic. However, there can no longer be any doubt that pharmacogenetics will be applied to clinical medicine with increasing breadth and depth, and that, ultimately, it will enhance our ability to individualize drug therapy.

Suggested Reading

Broder S, Venter JC. Sequencing the entire genomes of free-living organisms: the foundation of pharmacology in the new millennium. *Ann Rev Pharmacol Toxicol* 2000;40:97–132. *(Overview of genome sequencing and a primer on the possible implications of genetic diversity for pharmacology.)*

Drazen JM, Yandava CN, Dube L, et al. Pharmacogenetic association between *ALOX5* promoter genotype and the response to anti-asthma treatment. *Nat Med* 1999;22:168–171. *(Original study that showed different pharmacologic responses in people with different polymorphisms of the ALOX5 gene.)*

Evans WE, McLeod HL. Pharmacogenomics—drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348:538–549. *(Review describing the integration of genomics with pharmacogenetics.)*

Rieder MJ, Reiner AP, Gage BF, et al. Effect of *VKORC1* haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* 2005;352:2285–2293. *(Description of VKORC1 haplotypes, CYP2C9 genotypes, and their relationship to warfarin dose.)*

Wang L, Weinshilboum R. Thiopurine S-methyltransferase pharmacogenetics: insights, challenges and future directions. *Oncogene* 2006;25:1629–1638. *(Overview of the scientific development of a "classic" pharmacogenomic trait, with an emphasis on mechanisms linking genotype to phenotype.)*

Weinshilboum RM, Wang L. Pharmacogenetics and pharmacogenomics: development, science and translation. *Annu Rev Genomics Hum Genet* 2006;7:223–245. *(Review of pharmacokinetic and pharmacodynamic pharmacogenomic variation, as well as challenges to the translation of this science into the clinic.)*

Spotting the target: microarrays for disease gene discovery

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Microarray technologies enable genome-scale expression measurements. Already proved to be of value for the functional analysis of individual genes and biological processes, the application of expression profiling to disease gene discovery is now growing in importance and practicality.

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Abbreviations

CGH	comparative genomic hybridization
NF2	neurofibromatosis type 2
PARP	poly(ADP-ribose) polymerase
TD	Tangier disease

Introduction

Positional cloning projects have been greatly facilitated by the availability of increasingly precise maps and sequence databases for diverse species. This same avalanche of genomic data has inspired an intense effort to study aspects of genome function in a high-throughput fashion. The parallel analysis of gene expression has emerged as one of the most productive embodiments of this approach.

Practical technologies for large-scale gene-expression analysis are now being widely implemented. Microarrays comprising either oligonucleotides or cDNA fragments representing thousands of genes are well suited to the analysis of multiple samples [1,2]. To obtain genome-scale expression data, mRNA from the source of interest is converted to an appropriately labeled form and hybridized to the microarray. Both radioactive and fluorescence-detection strategies are in use to measure the resulting hybridization signal. The resulting raw data — an image obtained from a fluorescence scanner or phorphorimager — is processed with computer software to generate a spreadsheet of gene-expression values. The application of statistical techniques to microarray data allows classification and class discovery within a group of samples, and clustering of genes according to their pattern of expression.

Microarrays have been successfully applied to characterize biological processes and to dissect pathways downstream of a particular gene of interest. Studies in the yeast *Saccharomyces cerevesiae*, with its relatively small genome and highly tractable genetics, have led the way and continue with recent reports on signal transduction [3], meiosis [4] and transcript localization [5]. Despite the challenges posed by their genome sizes, large-scale expression analysis in mammals is also becoming increasingly productive.

As the technology for microarray analysis has matured and disseminated, new applications continue to be developed. One frequently discussed area is the potential use of microarray expression analysis in projects to positionally clone and discover disease genes. Although reviews of this topic outnumber reports of concrete achievement, it is appropriate to examine the state of the art and to consider how microarray analysis might accelerate these types of research. I discuss these points, together with recent developments in microarray research, in this review.

How might microarrays help find hereditary disease genes?

Several major approaches to locating hereditary disease genes might be imagined. In the simplest case, the target gene of interest might be identified directly by characteristic changes in expression level across a series of samples. Alternatively, statistical analysis of microarray data might aid gene discovery by revealing pathways related to the target gene and facilitating identification of candidate genes.

Microarrays can also be used to analyze genomic DNA rather than mRNA. This is illustrated by the special case of copy-number change in cancer, where it is possible to use array-format comparative genomic hybridization (CGH) to define genes associated with cancer progression [6••,7,8•]. In CGH, gene copy number is measured in a DNA sample labeled with one fluorochrome by comparison to the signal obtained by simultaneous hybridization of normal DNA labeled with a second fluorochrome. In principle, copy-number data can be linked to expression data to define a list of candidate target genes associated with gain of chromosomal regions [9•,10]. Although there is no example to date, tumor suppressors might be mapped by linking loss of gene expression to regions of deletion in tumors.

Of course, microarrays can be used as sophisticated dot blots to screen arrays of clones isolated with techniques such as RDA [11]. (RDA [representational difference analysis] is a PCR-based subtraction technique that can be used to isolate DNA fragments that vary in abundance between two sources.) Stephan *et al.* [12] have identified exons of the Niemann–Pick Type C disease isolated from arrayed genomic sequences using mRNA from cells differentially expressing *NPC1*. Finally, genes might be linked to specific phenotypes, particularly in yeast, through methods that allow genome-wide mutational screens using microarrays as a readout [13].

Finding the best candidate

It is enticing to hope that analysis of microarray data might lead to the direct identification of disease genes. Ideally, one would compare a group of samples of varying genotype and identify good candidate genes by their pattern of gene

expression. The expected signature of a mutant gene is reduced expression level in samples with the abnormal allele. For this strategy to work, the mutant allele would have to be either deleted or result in a poorly expressed transcript.

Fortunately, the phenomenon of nonsense-mediated decay of mRNA gives some reason to hope that this result might actually be achieved. Nonsense-mediated decay (reviewed in [14]) results in the degradation of certain mRNAs containing premature termination codons. This phenomenon has been observed in a number of disease genes [15,16].

In addition, abnormalities in 3′-untranslated region structure that interfere with normal polyadenylation may also lead to reduced survival of transcripts [17]. A reduction in steady-state mRNA levels of disease genes cannot be assumed, however, because the competence of a transcript to undergo nonsense-mediated decay is variable and some mutations may result in exon skipping [18,19], as has been shown by Liu *et al.* [20] for the *BRCA1* gene. This strategy also requires a sufficient number of samples from cells or tissues affected by the disease to help optimize the downstream data analysis.

Although obvious, an additional requirement of expression-based strategies is that the target gene is actually represented on the microarrays used. Although arrays of more than 10,000 genes are commonplace and complete genome microarrays can be anticipated, they are not yet routinely available. It is also probably unrealistic to assume that only a single gene or a few genes will stand out from the crowd with sufficient clarity to allow easy candidate selection. More likely, a strategy combining positional information with expression information will be necessary.

This combination of approaches has been used by Lawn *et al.* [21••] in the discovery of the Tangier disease (TD) gene *ABC1*. Microarray analysis led to the generation of a list of 175 cDNAs underexpressed by 2.5-fold or more in the fibroblasts of an affected individual. By combining this data with linkage information that localized the disease gene to chromosome 9q between the markers WI-14706 and WI-4062, the candidate list was narrowed sufficiently to identify the gene *ABC1*, which did indeed carry mutations.

Notably, Lawn *et al.* [21••] used commercial cDNA arrays containing 58,800 cDNAs, which presumably provided a reasonably thorough genome scan. One might imagine that regional searches could be made by constructing targeted microarrays covering a particular candidate region. This has been done for the X chromosome and for chromosome 17q [9•,22••].

It is important to bear in mind that almost all research employing microarray expression analysis depends heavily on statistical analysis to extract the most useful information from the huge number of data points generated. This means that any investigator attempting to use microarrays for disease gene discovery will also seek to go beyond this direct type of search and also examine the broader effects of mutation on gene expression in samples from affected individuals.

If one were not able to identify easily a candidate gene by virtue of its underexpression, perhaps the recognition of pathways altered consistently across a set of specimens might lead to the identification of good candidate genes or, at the very least, might illuminate some aspects of pathogenesis.

Finding the disease pathway affected by known genes

The complexity of microarray data is illustrated by another interesting feature of the TD data — the overexpression of 375 cDNAs by 2.5-fold or more. This result, revealing a total of 550 cDNAs with altered expression, is probably typical of what might be expected in most projects. In addition to innumerable technical factors, variations in gene expression across samples might be due to random fluctuations or confuting variables such as age, sex, site of sample and irrelevant genetic variations. Still, it would seem reasonable to suppose that the presence of a mutation in a pathway might frequently lead to secondary events affecting the level of expression of many other genes functionally connected to the disease gene.

Most published examples attempting to place genes from microarray data on samples carrying mutations into coherent pathways are in the setting of model systems for which the mutation is already known. McNeish *et al.* [23•] have examined a mouse model of TD with microarrays containing 11,000 genes and have identified 131 genes with greater than 1.8-fold differential regulation, many of which can be grouped into a few function-related categories. Their study demonstrates how studies of a relatively tractable experimental model can enhance the value of data obtained from human samples.

Likewise, Soukas *et al.* [24•] examined gene expression in white adipose tissue from mice expressing varying levels of the leptin gene. Seventy-seven genes were dysregulated by threefold or more in these *ob*/*ob* mice, including a number of key genes in fat metabolism. One cluster of genes was coordinately regulated by SREBP-1/ADD1, but the regulating mechanisms linking genes in several other clusters remain unknown. Although the complete pattern of changes observed cannot be explained as yet, the relevance of the leptin gene to fat metabolism is amply demonstrated.

Simbulan-Rosenthal *et al.* [25•] examined fibroblasts from mice deficient in poly(ADP-ribose) polymerase (PARP) with microarrays covering 11,000 genes and identified 91 genes differentially regulated by at least twofold relative to wild-type fibroblasts. About 40% of these could be related to either the cell cycle or remodeling of the cytoskeleton or extracellular matrix — processes known to be associated with PARP function.

Callow *et al.* [26] examined livers from apolipoprotein AI knockout mice, scavenger receptor B1 transgenic mice and wild-type mice on microarrays containing 5600 cDNAs. They used *t*-test statistics to identify a small number of genes that differed significantly across these conditions.

For disease gene discovery, the interpretation of expression data in terms of pathways is more difficult because there is no *a priori* knowledge of the disease gene function. This leads to a consideration of the process of grouping differentially expressed genes into pathways.

Placing genes in pathways to gain clues about unknown genes

Can pathways actually be discerned from microarray data? It is worthwhile considering some of the individual steps in the process of deducing pathway information from these data. Clustering of genes into co-regulated groups is computationally straightforward and readily generates this type of information [27]. Similarly, there has been great success in classifying biological samples from microarray data, particularly for cancer specimens [28•,29••–31••,32]. These studies are promising in identifying critical genes for cancer progression at the expression level, although these are not necessarily 'disease genes' in the genetic sense [33••].

Nonetheless, Hedenfalk *et al.* [34••] have even shown that it is possible to sort breast cancer specimens according to the presence of hereditary mutations in *BRCA1* or *BRCA2*. One of the most striking results in their study was the demonstration that a sample that clustered with those from patients carrying mutations in *BRCA1* lacked a *BRCA1* mutation but was highly methylated at the *BRCA1* promoter.

It might be hoped that this approach could aid complex disease gene discovery by sorting samples into groups that share a common genetic defect. When combined with positional data from linkage analysis, such an approach might be expected to take on a significant role in the study of complex disease.

In contrast to clustering samples and genes, the interpretation of expression data to infer the pathway affected by a disease gene mutation is much more problematic. The initial problem one faces in this type of analysis is the limited annotation of the genome. When examining an expression database, one immediately encounters difficulty in placing genes into functional categories. This is beset with a number of obstacles, the first of which are the numerous aliases that confuse gene nomenclature.

The introduction of two on-line resources, LocusLink and Refseq, have gone a long way towards overcoming this problem by providing a unique identifier and curated sequence for each gene [35]. This is absolutely critical to the next phase of analysis, which is the cross-reference to other databases of gene function including, most importantly, literature databases. Frequently, different functions or interpretations of gene function are linked to distinct aliases for a given gene. Only by thoroughly combing the literature, can the most comprehensive picture of gene function be obtained. Substantial efforts are being made to organize the genes of known function into meaningful categories.

Although a detailed discussion of the problem of gene annotation is beyond the scope of this review, the public availability of certain resources should be noted. In particular, the Gene Ontology consortium uses a common language to organize functional information in all species [36]. Currently, the Gene Ontology database contains database links for *Drosophila*, *S. cerevesiae*, mouse and *Caenorhabditis elegans*. Genes are categorized in three hierarchical schemes according to molecular function, biological process and cellular component.

Methods to process groups of genes with respect to literature databases are also under development [37–39]. One system, High-density Array Pattern Interpreter (HAPI; http://array.ucsd.edu/hapi/), is publicly available. It is anticipated that search engines that can carry out these computations with the output of expression databases will significantly accelerate the process of organizing data from microarrays.

Although it is relatively straightforward to identify lists of genes that are co-regulated across a set of samples, this may not be a sufficiently sensitive method to extract functionally related genes. Intensive efforts to establish alternate computational methods are continuing.

Seungchan *et al.* [40] have described a multivariate technique that has the potential to identify relationships among genes that are refractory to methods based on linear correlation. Akutsu *et al.* [41] have proposed a method for modeling gene expression in terms of Boolean networks, whereas Friedman *et al.* [42] have proposed a Bayesian method. Hastie *et al.* [43] have described a method termed 'gene shaving', which differs from hierarchical clustering in that genes may belong to more than one cluster. Brown *et al.* [44] have advocated the use of method based on the theory of 'support vector machines', a computer learning method that they have adapted to the functional categorization of expression data.

Finding regulatory motifs

One great challenge remaining in the analysis of mammalian expression data will be to link this information to regulatory elements in the genome sequence. Promising results in yeast continue to appear. Iyer *et al.* [45••] have taken advantage of the small size of the yeast genome to array non-coding DNA and identify the genes regulated by the cell-cycle transcription factors SBF and MBF. Ren *et al.* [46••] have achieved similar results for Gal4 and Ste12, and Livesey *et al.* [47] have identified the response element configuration and genes responsive to the mouse homeobox gene *Crx*.

The development of progressively more sophisticated computational methods increases optimism that genes related to a phenotype can be accurately extracted and placed in functionally related groups to help generate new hypotheses. Even with this goal accomplished, one would expect that the effects of mutation on one biochemical pathway will radiate to affect numerous other pathways. Identifying the pathway primarily affected will be a significant challenge.

Using microarrays to map genomic DNA

Although using microarrays to identify regions of copy-number change in cancers has received the most attention, array format CGH might also be useful for mapping hereditary disease genes. Bruder *et al.* [48••] have used microarrays tiled across a 7-Mb region including the neurofibromatosis type 2 gene (NF2) to analyze DNA from 116 NF2 patients. Using this exquisitely accurate system, they were able to identify 24 patients with gene deletions and show that there was no correlation with disease severity.

In principle, this type of approach could be applied to a region containing an unknown disease gene. Because positional cloners frequently assemble contigs covering regions of linkage, the availability of genomic clones may not be problematic. However, the technology for arraying and accurately determining copy number in this setting is still confined to a few laboratories.

Conclusions

Unquestionably, large-scale expression analysis is now established in the study of genome function. The power of this approach continues to be enhanced by technical advances and, importantly, by the development of very large coherent expression databases from samples collected across a broad range of conditions [49••]. The recent report from Shoemaker *et al.* [50] points to the future with microarrays composed of over one million oligonucleotides representing 442,785 exons predicted from the draft human genome sequence. These developments suggest that microarray analysis will increasingly merit consideration as an ancillary technique to facilitate hereditary disease gene discovery.

Update

Loftus and Pavan have recently used melanocyte-specific microarrays to identify a mouse coat color gene (S Loftus, W Pavan, personal communication).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Schena M, Shalon D, Davis RW, Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995, 270:467-470.

2. Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SP: Accessing genetic information with high-density DNA arrays. *Science* 1996, 274:610-614.

3. Roberts CJ, Nelson B, Marton MJ, Stoughton R, Meyer MR, Bennett HA, He YD, Dai H, Walker WL, Hughes TR *et al.*: Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. *Science* 2000, 287:873-880.

4. Primig M, Williams RM, Winzeler EA, Tevzadze GG, Conway AR, Hwang SY, Davis RW, Esposito RE: The core meiotic transcriptome in budding yeasts. *Nat Genet* 2000, 26:415-423.

5. Takizawa PA, DeRisi JL, Wilhelm JE, Vale RD: Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. *Science* 2000, 290:341-344.

6. •• Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO: Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat Genet* 1999, 23:41-46.

This study shows that cDNA microarrays can be used to determine gene copy number by comparative genomic hybridization. Using the same array for copy number and expression measurements enables rapid mapping of regions of gene amplification in cancers. It also raises the possibility for finding mutational targets associated with copy-number loss and reduced expression.

7. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y *et al.*: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998, 20:207-211.

8. • Mei R, Galipeau PC, Prass C, Berno A, Ghandour G, Patil N, Wolff RK, Chee MS, Reid BJ, Lockhart DJ: Genome-wide detection of allelic imbalance using human SNPs and high-density DNA arrays. *Genome Res* 2000, 10:1126-1137.

An oligonucleotide microarray designed to cover 600 SNPs yields allelic imbalance data on about 150 markers in analyses of tumor-cell DNA. However, the method cannot distinguish between loss and gain at a locus.

9. • Barlund M, Monni O, Kononen J, Cornelison R, Torhorst J, Sauter G, Kallioniemi O-P, Kallioniemi A: Multiple genes at 17q23 undergo amplification and overexpression in breast cancer. *Cancer Res* 2000, 60:5340-5344.

This study shows the potential of tissue microarrays to establish rapidly the rate of gene amplification at a series of loci on 17q23 in 372 breast cancers. This is an excellent illustration of the complementary application of tissue and expression microarrays.

10. Virtaneva K, Wright FA, Tanner SM, Yuan B, Lemon WJ, Caligiuri MA, Bloomfield CD, de La Chapelle AA, Krahe R: Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. *Proc Natl Acad Sci USA* 2001, 98:1124-1129.

11. Welford SM, Gregg J, Chen E, Garrison D, Sorensen PH, Denny CT, Nelson SF: Detection of differentially expressed genes in primary tumor tissues using representational differences analysis coupled to microarray hybridization. *Nucleic Acids Res* 1998, 26:3059-3065.

12. Stephan DA, Chen Y, Jiang Y, Malechek L, Gu JZ, Robbins CM, Bittner ML, Morris JA, Carstea E, Meltzer PS *et al.*: Positional cloning utilizing genomic DNA microarrays: the Niemann-Pick type C gene as a model system. *Mol Genet Metab* 2000, 70:10-18.

13. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H *et al.*: Functional characterization of the ***S. cerevisiae*** genome by gene deletion and parallel analysis. *Science* 1999, 285:901-906.

14. Frischmeyer PA, Dietz HC: Nonsense-mediated mRNA decay in health and disease. *Hum Mol Genet* 1999, 8:1893-1900.

15. Christiano AM, Amano S, Eichenfield LF, Burgeson RE, Uitto J: Premature termination codon mutations in the type VII collagen gene in recessive dystrophic epidermolysis bullosa result in nonsense-mediated mRNA decay and absence of functional protein. *J Invest Dermatol* 1997, 109:390-394.

16. Jacoby LB, MacCollin M, Parry DM, Kluwe L, Lynch J, Jones D, Gusella JF: Allelic expression of the NF2 gene in neurofibromatosis 2 and schwannomatosis. *Neurogenetics* 1999, 2:101-108.

17. Muhlrad D, Parker R: Aberrant mRNAs with extended 3′ UTRs are substrates for rapid degradation by mRNA surveillance. *RNA* 1999, 5:1299-1307.

18. Bauer JW, Rouan F, Kofler B, Rezniczek GA, Kornacker I, Muss W, Hametner R, Klausegger A, Huber A, Pohla-Gubo G *et al.*:

A compound heterozygous one amino-acid insertion/nonsense mutation in the plectin gene causes epidermolysis bullosa simplex with plectin deficiency. *Am J Pathol* 2001, 158:617-625.

19. Romao L, Inacio A, Santos S, Avila M, Faustino P, Pacheco P, Lavinha J: Nonsense mutations in the human β-globin gene lead to unexpected levels of cytoplasmic mRNA accumulation. *Blood* 2000, 96:2895-2901.

20. Liu HX, Cartegni L, Zhang MQ, Krainer AR: A mechanism for exon skipping caused by nonsense or missense mutations in BRCA1 and other genes. *Nat Genet* 2001, 27:55-58.

21. •• Lawn RM, Wade DP, Garvin MR, Wang X, Schwartz K, Porter JG, Seilhamer JJ, Vaughan AM, Oram JF: The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J Clin Invest* 1999, 104:R25-R31.
This study illustrates the potential of using gene-expression analysis in concert with positional information to identify a hereditary disease gene.

22. •• Sudbrak R, Wieczorek G, Nuber UA, Mann W, Kirchner R, Erdogan F, Brown CJ, Wohrle D, Sterk P, Kalscheuer VM *et al.*: X chromosome-specific cDNA arrays: identification of genes that escape from X-inactivation and other applications. *Hum Mol Genet* 2001, 10:77-83.
The authors construct a specialized microarray containing 2423 cDNAs from the X chromosome. They confirm its utility with tests on samples containing varying numbers of X chromosomes. Notably, they can identify three genes contained within a male-viable deletion.

23. • McNeish J, Aiello RJ, Guyot D, Turi T, Gabel C, Aldinger C, Hoppe KL, Roach ML, Royer LJ, de Wet J *et al.*: High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc Natl Acad Sci USA* 2000, 97:4245-4250.
The authors use gene-expression analysis in a mouse knockout model of Tangier disease. This study provides an example of the progress and problems in identifying pathways downstream of disease genes. It also illustrates the advantages of studying a disease model in the mouse for which target tissues can be readily obtained.

24. • Soukas A, Cohen P, Socci ND, Friedman JM: Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* 2000, 14:963-980.
This study demonstrates that a large proportion of the genes altered in mice carrying mutated leptin genes can indeed be placed in pathways credibly related to leptin function.

25. • Simbulan-Rosenthal CM, Ly DH, Rosenthal DS, Konopka G, Luo R, Wang ZQ, Schultz PG, Smulson ME: Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase. *Proc Natl Acad Sci USA* 2000, 97:11274-11279.
This study explores the altered patterns of gene expression in mice deficient for poly(ADP-ribose) polymerase, and links the observed changes to pathways affecting cell cycle, DNA replication, the extracellular matrix and the cytoskeleton.

26. Callow MJ, Dudoit S, Gong EL, Speed TP, Rubin EM: Microarray expression profiling identifies genes with altered expression in HDL-deficient mice. *Genome Res* 2000, 10:2022-2029.

27. Eisen MB, Spellman PT, Brown PO, Botstein D: Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 1998, 95:14863-14868.

28. • Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, Iyer V, Jeffrey SS, Van de Rijn M, Waltham M *et al.*: Systematic variation in gene expression patterns in human cancer cell lines. *Nat Genet* 2000, 24:227-235.
The authors show that expression profiles determined by cDNA microarrays analysis can be used to cluster a variety of cancer cell lines into distinct disease categories.

29. •• Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X *et al.*: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000, 403:503-511.
By using a cDNA microarray (the 'lymphochip') optimized for relevant gene content, the authors are able to subclassify diffuse large B-cell lymphoma specimens into two groups. Of importance, they can relate these groups to normal B-cell differentiation and show that the group of patients with a germinal-center-like phenotype has a better prognosis.

30. •• Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, Radmacher M, Simon R, Yakhini Z, Ben-Dor A *et al.*: Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 2000, 406:536-540.
In this study, the potential of microarrays for class discovery is demonstrated by the identification of an unknown subset of melanoma samples with a characteristic gene-expression profile. By comparing this profile with that of an *in vitro* model of melanoma metastasis, the authors can predict biological properties (such as reduced motility) associated with this subset, which they confirm experimentally.

31. •• Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA *et al.*: Molecular portraits of human breast tumours. *Nature* 2000, 406:747-752.
The authors use cDNA microarrays to establish the gene-expression profile of breast cancer specimens. The results are striking and suggest distinct histogenesis for subsets of breast cancers. Along with Bittner *et al.* [30••], this study shows that two samples from the same patient tend to a greater similarity than any pair within a sample set.

32. Amler LC, Agus DB, LeDuc C, Sapinoso ML, Fox WD, Kern S, Lee D, Wang V, Leysens M, Higgins B *et al.*: Dysregulated expression of androgen-responsive and nonresponsive genes in the androgen-independent prostate cancer xenograft model CWR22-R1. *Cancer Res* 2000, 60:6134-6141.

33. •• Clark EA, Golub TR, Lander ES, Hynes RO: Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* 2000, 406:532-535.
This paper shows the power of parallel gene-expression analysis to identify critical pathways and genes, in this case related to metastasis. RhoC is identified as a critical regulator of tumor-cell invasion in an *in vivo* model, an observation confirmed by expressing RhoC constructs.

34. •• Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi OP *et al.*: Gene-expression profiles in hereditary breast cancer. *N Engl J Med* 2001, 344:539-548.
The authors investigate hereditary breast cancers arising in patients with *BRCA1* or *BRCA2* mutations to determine whether they have a characteristic gene-expression profile in comparison to sporadic tumors. This proves to be the case. *BRCA1* tumors cluster together particularly strongly. A sporadic case that clusters in this group has methylation of the *BRCA1* promoter and low expression of *BRCA1*. In addition to providing clues to the pathogenesis of hereditary breast cancer, these observations raise the possibility that gene-expression profiling may be used to classify tissue samples in complex diseases to aid gene discovery.

35. Pruitt KD, Maglott DR: RefSeq and LocusLink: NCBI gene-centered resources. *Nucleic Acids Res* 2001, 29:137-140.

36. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT *et al.*: Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000, 25:25-29.

37. Shatkay H, Edwards S, Wilbur WJ, Boguski M: Genes, themes and microarrays: using information retrieval for large- scale gene analysis. *Proc Int Conf Intell Syst Mol Biol* 2000, 8:317-328.

38. Stapley BJ, Benoit G: Biobibliometrics: information retrieval and visualization from co-occurrences of gene names in Medline abstracts. *Pac Symp Biocomput* 2000:529-540.

39. Jenssen TK, Vinterbo S: A set-covering approach to specific search for literature about human genes. *Proc AMIA Symp* 2000:384-388.

40. Seungchan K, Dougherty EK, Chen Y, Krishnamoorthy S, Meltzer P, Trent JM, Bittner M: Multivariate measurement of gene expression relationships. *Genomics* 2000, 67:201-209.

41. Akutsu T, Miyano S, Kuhara S: Algorithms for identifying Boolean networks and related biological networks based on matrix multiplication and fingerprint function. *J Comput Biol* 2000, 7:331-343.

42. Friedman N, Linial M, Nachman I, Pe'er D: Using Bayesian networks to analyze expression data. *J Comput Biol* 2000, 7:601-620.

43. Hastie T, Tibshirani R, Eisen MB, Alizadeh A, Levy R, Staudt L, Chan WC, Botstein D, Brown P: 'Gene shaving' as a method for identifying distinct sets of genes with similar expression patterns. *GenomeBiology.com* 2000, 1.

44. Brown MP, Grundy WN, Lin D, Cristianini N, Sugnet CW, Furey TS, Ares M Jr, Haussler D: Knowledge-based analysis of microarray gene expression data by using support vector machines. *Proc Natl Acad Sci USA* 2000, 97:262-267.

45. •• Iyer VR, Horak CE, Scafe CS, Botstein D, Snyder M, Brown PO: Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. *Nature* 2001, 409:533-538.
This study is an important step towards using microarrays to map regulatory elements in yeast. The authors take advantage of the complete sequence to

construct arrays that allow them to identify 200 putative new targets for the transcription factors SBF and MBF.

46. •• Ren B, Robert F, Wyrick JJ, Aparicio O, Jennings EG, Simon I, Zeitlinger J, Schreiber J, Hannett N, Kanin E *et al.*: Genome-wide location and function of DNA binding proteins. *Science* 2000, 290:2306-2309.

As in Iyer *et al.* [45••], the authors use microarrays to identify yeast genes directly regulated by two transcription factors, Gal4 and Ste12. Expression arrays lend themselves to identifying patterns of co-regulation, and these two studies take the next step of linking this information to genomic sequence.

47. Livesey FJ, Furukawa T, Steffen MA, Church GM, Cepko CL: Microarray analysis of the transcriptional network controlled by the photoreceptor homeobox gene Crx. *Curr Biol* 2000, 10:301-310.

48. •• Bruder CE, Hirvela C, Tapia-Paez I, Fransson I, Segraves R, Hamilton G, Zhang XX, Evans DG, Wallace AJ, Baser ME *et al.*: High resolution deletion analysis of constitutional DNA from neurofibromatosis type 2 (NF2) patients using microarray-CGH. *Hum Mol Genet* 2001, 10:271-282.

This study shows the outstanding quantitative data that can be obtained with array-format comparative genomic hybridization when large-insert genomic clones are arrayed. The authors are able to map constitutional deletions in the *NF2* locus with high resolution and precision. The potential of this approach for disease gene discovery is evident.

49. •• Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD *et al.*: Functional discovery via a compendium of expression profiles. *Cell* 2000, 102:109-126.

Although this study involves expression data from yeast, it provides a valuable model for the integration of data from 300 conditions. Using this database, the authors can place eight anonymous genes into distinct functional pathways.

50. Shoemaker DD, Schadt EE, Armour CD, He YD, Garrett-Engele P, McDonagh PD, Loerch PM, Leonardson A, Lum PY, Cavet G *et al.*: Experimental annotation of the human genome using microarray technology. *Nature* 2001, 409:922-927.

6. Food and Drug Administration. *FDA Approves restricted marketing of Lotronex*. <http://www.fda.gov/bbs/topics/NEWS/2002/NEW00814.html> (Department of Health and Human Services, Washington, 2002).
7. Houn, F. Letter, NDA-21-107/S-005, Office of Drug Evaluation II, Centre for Drug Evaluation and Research <http://www.fda.gov/cder/foi/appletter/2002/21107s5ltr.pdf> (Food and Drug Administration to GlaxoSmithKline, 2002).
8. Goldstein, D. B. Pharmacogenetics in the laboratory and the clinic. *N. Engl. J. Med.* **348**, 553–556 (2003).
9. Holtzman, N. A. in *Pharmacogenomics: Social, Ethical, and Clinical Dimensions* (ed. Rothstein, M. A.) 163–186 (Wiley-Liss, Hoboken, New Jersey, 2003).
10. Omenn, G. & Motulsky, A. in *Pharmacogenomics: Social, Ethical, and Clinical Dimensions* (ed. Rothstein, M. A.) 137–162 (Wiley-Liss, Hoboken, New Jersey, 2003).
11. Nuffield Council of Bioethics. *Pharmacogenetics: Ethical Issues* (Nuffield Council of Bioethics, London, 2003).
12. Melzer, D. *et al. My Very Own Medicine: What Must I Know?* <http://www.phpc.cam.ac.uk/epg/Report.pdf> (Department of Public Health and Primary Care, Univ. Cambridge, Cambridge, 2003).
13. Lindpaintner, K. Pharmacogenetics and the future of medical practice. *Br. J. Clin. Pharmacol.* **54**, 221–230 (2002).
14. Pirmohamed, M. & Park, B. K. Genetic susceptibility to adverse drug reactions. *Trends Pharmacol. Sci.* **22**, 298–305 (2001).
15. Roses, A. Pharmacogenetics and the practice of medicine *Nature*, **405**, 857–865 (2000).
16. Clozapine Summary Sheet SS95/09 <www.keele.ac.uk/depts/mm/MTRAC/ProductInfo/summaries/C/CLOZAPINEs.html> (MTRAC, Department of Medicines Management, Keele Univ, Keele, 1995)
17. Arranz, M. J. *et al.* Pharmacogenetic prediction of clozapine response. *Lancet* **355**, 1615–1616 (2000).
18. Mancama, D., Arranz, M. J. & Kerwin, R. W. Genetic predictors of therapeutic response to clozapine: current status of research. *CNS Drugs* **16**, 317–324 (2002).
19. Holtzman, N. A. & Watson, M. S. (eds) *Promoting Safe and Effective Genetic Testing In the United States*. Final Report. (Johns Hopkins Univ. Press, Baltimore, Maryland, 1998).
20. Robertson, J. A., Brody, B., Buchannan, A., Kahn, J. & McPherson, E. Pharmacogenetic challenges for the health care system. *Health Aff.* **21**, 155–167 (2002).
21. Rothstein, M. A. & Epps, P. G. Ethical and legal implications of pharmacogenomics. *Nature Rev. Genet.* **2**, 228–231 (2001).
22. Issa, A. M. Ethical perspectives on pharmacogenomic profiling in the drug development process. *Nature Rev. Drug Discov.* **1**, 300–308 (2002).
23. Smart, A., Parker, M. & Martin, P. Tailored medicine: who will it fit? The ethics of patient and disease stratification. *Bioethics* (In the press).
24. Pirmohamed, M & Lewis, G. in *Regulating Pharmaceuticals in Europe: Striving For Efficiency, Equity and Quality* (eds Mossialos, E., Mrazek, M. & Walley, T.) 279–296 (Open Univer. Press, Maidenhead, 2004).
25. Stearns, V., Davidson, N. E. & Flockhart, D. Pharmacogenetics in the treatment of breast cancer. *Pharmacogenetics* **4**, 143–153 (2004).
26. Marsh, S. & McLeod, H. Cancer pharmacogenetics. *Brit. J. Cancer* **90**, 8–11 (2004).
27. Department of Health and Human Services. Draft guidance for industry: pharmacogenomic data submissions <http://www.fda.gov/OHRMS/DOCKETS/98fr/03d-0497-nad0001vol1.pdf> (Food and Drug Administration, 2003).
28. Savage, D. R. FDA guidance on pharmacogenomics data submission. *Nature Rev. Drug Discov.* **2**, 937–938 (2003).
29. The European Agency for the Evaluation of Medicinal Products. Concept paper on pharmacogenetics — briefing matters. CPMP/4445/03 <http://www.emea.eu.int/pdfs/human/pharmacogenetics/444503en.pdf> (The European Agency for the Evaluation of Medicinal Products, 23 Jan 2003).
30. Lesko, L. J. & Woodcock, J. Pharmacogenomic-guided drug development: regulatory perspective. *Pharmacogenomics J.* **2**, 20–24 (2002).
31. Ratner, M. L. Pharmacogenomic data and labeling: a less-safe harbor for existing drugs? *Windhover's Update* <http://www/windhover.com/update/05252003/20038000094> (2003).
32. Anonymous. FDA's Lesko says pharmacogenomics guidance paper due mid-year. *Pharmacogenomics Reporter (New York)* 6 (17 Jan 2003).
33. Abraham, J. & Lewis, G. *Regulating Medicines in Europe* (Routledge, London, 2000).
34. European Commission. The *in vitro* diagnostic medical devices directive (98/79/EC). *Official J. Eur. Communities* L331/1 (27 Oct 1998).
35. Danzon, P. & Towse, A. The economics of gene therapy and of pharmacogenetics. *Value Health* **5**, 5–13 (2002).
36. Audit Commission. A spoonful of medicine: medicines management in NHS hospitals. <http://www.audit-commission.gov.uk/Products/NATIONAL-REPORT/E83C8921-6CEA-4b2c-83E7-F80954A80F85/nrspoonfulsugar.pdf> (Audit Commission, London, 2001).

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Competing interests statement
The authors declare no competing financial interests.

Online links

FURTHER INFORMATION
Encyclopedia of Life Sciences: http://www.els.net/pharmacogenetics
Institute for the Study of Genetics, Biorisks and Society: http://www.nottingham.ac.uk/igbis
Lewis' homepage: http://www.york.ac.uk/org/satsu/Staff/graham/graham.htm
Oxford Genetics Knowledge Park: http://www.oxfordgkp.org
Pharmacogenetics research at SATSU: http://www.york.ac.uk/res/pgx
University of York Science and Technology Studies Unit: www.york.ac.uk/org/satsu
Webster's homepage: http://www.york.ac.uk/depts/soci/s_webs.html
Access to this interactive links box is free online.

TIMELINE

Pharmacogenetics – five decades of therapeutic lessons from genetic diversity

Urs A. Meyer

Abstract | Physicians have long been aware of the subtle differences in the responses of patients to medication. The recognition that a part of this variation is inherited, and therefore predictable, created the field of pharmacogenetics fifty years ago. Knowing the gene variants that cause differences among patients has the potential to allow 'personalized' drug therapy and to avoid therapeutic failure and serious side effects.

Pharmacogenetics (PGx) deals with genetically determined variation in how individuals respond to drugs . Observations implying that genetic variation was responsible for the diversity in some drug responses were already being made five decades ago. We now know that the therapeutic failure of drugs as well as serious adverse side effects of drugs on individuals or subpopulations of patients can both have a genetic component. The toll that such variation takes in terms of individual suffering, high healthcare costs, and even lives, is increasingly being recognized. Recent developments in genomics, and associated technological innovations, have invigorated the study of such variation. Pharmacogenetic research has seen an explosion of interest by physicians, geneticists and the pharmaceutical industry — as reflected in the rapid increase in the number of publications that contain this term (FIG. 1). PGx has the potential to identify the particular drug and the dose of drug that is most likely to be effective and safe for each patient. This has become one of the main goals of modern drug therapy, and is frequently described as 'personalized medicine'. But in spite of its importance in explaining the diversity of responses to drugs, the integration of PGx into clinical practice has met considerable challenges.

The history of PGx reflects the evolution of human genetics and genomics, of molecular pharmacology and modern drug therapy. The field has had its visionaries and godfathers, who realized its importance early in its history. These early pioneers laid the foundations for the landmark discoveries that form the basis of present concepts and approaches (TIMELINE).

The gestation of a discipline

Sir Archibald Garrod, the perceptive physician-scientist. Around the year 1898 the British physician Archibald Garrod was interested in urinary pigments and studied patients at St. Bartholomew's Hospital in London that had ALCAPTONURIA (see Glossary) and patients that had PORPHYRIA that was caused by sulphonal (a hypnotic) [1,2]. Garrod was probably the first to realize the inherited predisposition of certain individuals to alcaptonuria[1] and other conditions. In particular,

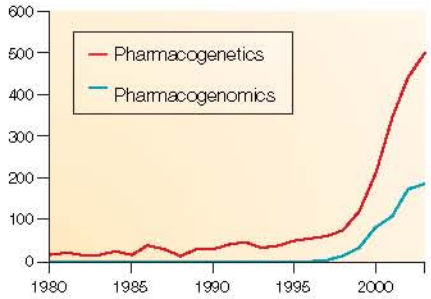


Figure 1 | **Appearance of the terms pharmacogenetics and pharmacogenomics in publications in PUBMED (National Library of Medicine).** Vogel first used the term pharmacogenetics (PGx) in 1959 (REF. 13). Publications on PGx have increased sharply in the last 5 years with the emergence of molecular genetics and genotyping technologies in clinical investigations. The term 'pharmacogenomics' first appeared in 1998.

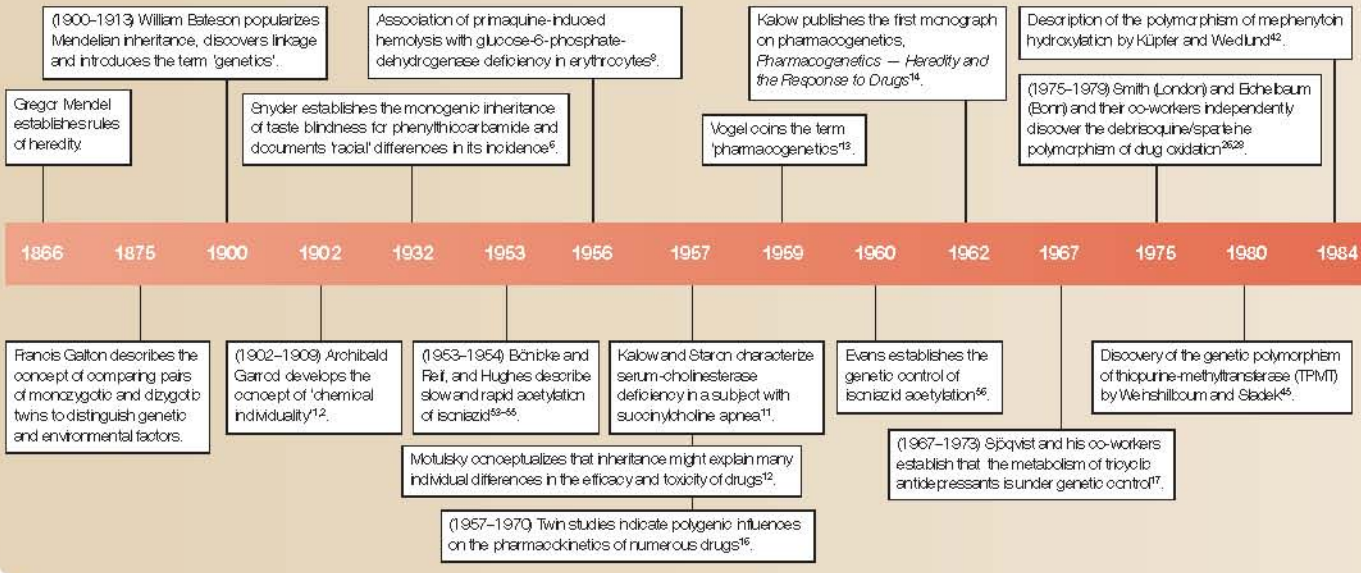
With particular foresight Garrod went on to develop the concept of CHEMICAL INDIVIDUALITY in man, which was first presented in the Croonian Lectures around 1908 and then in *The Inborn Errors of Metabolism*[2] and in *The Inborn Factors of Disease*[4] (FIG. 2). His amazingly prescient vision of the basic tenets of PGx is summarized in the following passage: "Even against chemical poisons taken by mouth, or by other channels, there are some means of defence. Every active drug is a poison, when taken in large enough doses; and in some subjects a dose which is innocuous to the majority of people has toxic effects, whereas others show exceptional tolerance of the same drug. Some chemical poisons are destroyed in the tissues, provided that the dose given be not too large, and others are combined up with substances to hand, and so rendered innocuous and got rid of."[4].

Figure 2 | **Sir Archibald E. Garrod (1958–1936).** Photograph taken by Simon Garrod in 1934. Modified from the cover of the monograph by Charles R. Scriver and Barton Childs on Garrod's book *The Inborn Factors Of Disease*[4]. Garrod coined the term 'chemical individuality'. Image reproduced with permission from REF. 63 © (1989) Oxford University Press.

Garrod observed that parental consanguinity was more common than usual among parents of children with alcaptonuria. Lore has it that Garrod recognized the Mendelian inheritance of alcaptonuria as a monogenic trait. This is unlikely, however, because Garrod was apparently unaware of the relationship between his observations and Mendel's work. It was probably William Bateson[3], another biologist who was ahead of his time, who interpreted Garrod's reports as recessive inheritance when he popularized Mendelian genetics in Britain. Bateson discovered genetic linkage and, in fact, introduced the term 'genetics' between 1902 and 1913.

Taste blindness: the first example of a pharmacogenetic study. Despite Garrod's insightful observation, the first experimental study that was of relevance to PGx was not of individual responses to a therapeutic drug: rather, it was of variation in the ability to taste a foreign chemical; specifically the inability to taste ('taste blindness' for) phenylthiocarbamide (PTC). While synthesizing chemicals in search for a sugar substitute, A. L. Fox[5] observed that some people detected the bitter taste of PTC when the crystals were placed on the tongue, whereas other people could detect only a very slight taste, or said it had the 'taste of sand'. L. H. Snyder[6], in an incredibly large study of 800 families, noted that this taste blindness was inherited in an autosomal-recessive fashion and that the frequency of non-tasters varied in populations of different ethnic origin or race. The molecular mechanism of 'taste blindness' for PTC and related substances — for example, propyl thiouracil — is unknown. Nonetheless, the study of 'taste blindness' was the prototype for future studies of pharmacogenetic variation. It was the first study of a common genetic polymorphism and it documented the association of race or ethnicity to a human response to chemicals.

Timeline | **A history of pharmacogenetics**



Landmark discoveries in the 1950s. The 1950s was the decade in which PGx emerged as a distinct discipline. New techniques allowed the more accurate measurement of enzyme activities, drug metabolites and drug responses. Although their significance was not recognised until late in the decade (see below), there were three clear examples of genetically determined variations in enzyme activity being shown to underlie adverse drug reactions, as Garrod predicted.

Alf Alving and co-workers observed that, in World War II, approximately 10% of African-American soldiers, but only a very small number of Caucasian soldiers, developed acute haemolytic crises when given an average dose of primaquine or other chemically related antimalarial drugs[7]. It was later shown that this sensitivity was caused by a deficiency of glucose 6-phosphate dehydrogenase (G6PD), which altered erythrocyte metabolism[8]. The same genetic defect was suspected to account for Favism — haemolysis after ingestion of Fava beans — which occurred in some individuals of Mediterranean descent. We now know that the *G6PD* locus on chromosome X is one of the most polymorphic genetic sites in humans. More than 400 million people carry one of 135 definitive *G6PD* variants and are at risk of haemolysis when exposed to drugs. The frequency of low-activity alleles of *G6PD* is highly correlated with the prevalence of malaria[9].

The drug succinylcholine (also known as suxamethonium), which is administered as an adjunct to anaesthesia, provided the second example of a genetic variant that causes an abnormal drug response — in this case prolonged APNEA[10]. Succinylcholine causes muscular paralysis, usually for a few minutes, but in exceptional cases for up to one hour. The prolonged effect is due to altered kinetics of a pseudocholinesterase (butyrylcholinesterase). Family studies showed that PSEUDOCHOLINESTERASE DEFICIENCY was inherited as an autosomal-recessive trait[11].

Perhaps the most well-known example of a genetic defect in drug biotransformation is the acetylation polymorphism. It was first observed with the advent of ISONIAZID therapy in tuberculosis in the 1950s and was shown 40 years later to be due to mutations in *N*-acetyltransferase-2 (*NAT2*) on chromosome 8 (BOX 1).

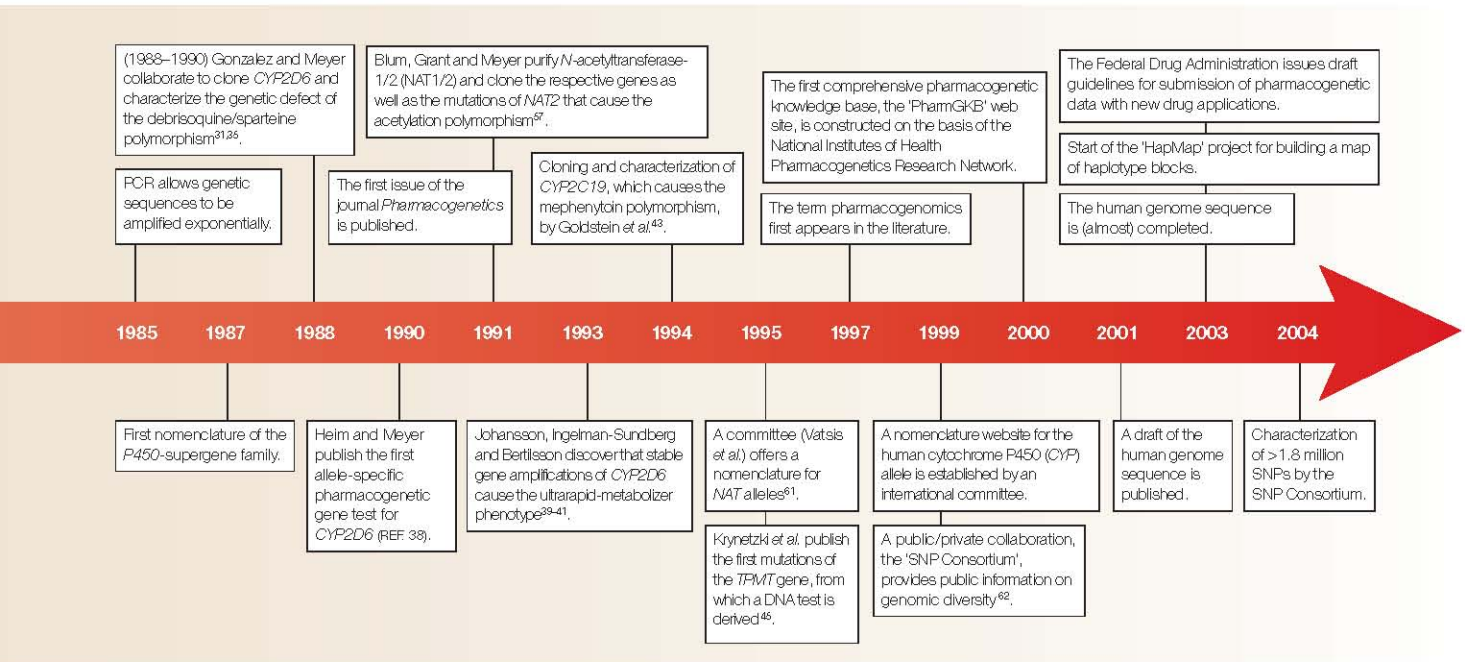
Box 1 | Acetylation polymorphism: prototype of variability in drug metabolism

Isoniazid, which was introduced in 1952, was the first drug that was effective in the treatment of tuberculosis. Bönike and Reif[53] in Germany, and Hughes in the United States[54] observed that, for any individual, urinary excretion of unchanged isoniazid was constant on repeated administration, but that there were marked differences in excretion among individuals. In later studies, Hughes determined that the differences were due to differences in the individual's ability to convert isoniazid to acetylisoniazid. 'Slow acetylators' were more prone to suffer from isoniazid toxicity, that is, PERIPHERAL NEUROPATHY[55]. Family studies showed this was an autosomal recessive trait. Subsequently, measurement of the plasma concentration of isoniazid 6 hours after an isoniazid dose allowed Evans[56] to identify two groups, rapid and slow acetylators.

These initial studies triggered many epidemiological, pharmacological and clinical studies in numerous countries, and provided a model of how pharmacogenetic traits could be analyzed. The acetylation polymorphism that was identified also affects the disposition of a variety of other drugs, including sulphonamides, dapsone (Avlosulfon; Wyeth Ayerst), phenelzine (Nardil; Pfizer Inc.), hydralazine, procainamide and numerous other foreign chemicals including chemical carcinogens. However it was not until 40 years later that the molecular causes of this polymorphism were elucidated[57,58]. Cloning of the cDNA that encodes the cytosolic enzyme *N*-acetyltransferase-2 in 1991 allowed identification of 2 common alleles[57]. These two alleles, were identified as restriction-fragment length polymorphisms and correspond to alleles now called *NAT2*5* and *NAT2*6*. These variants account for over 90% of slow-acetylator alleles[24]. As of June 2003, 14 mutations in the coding region of *NAT2* were described, which — alone or in various combinations — produce 36 different alleles (see Arylamine *N*-Acetyltransferase (NAT) Nomenclature in the online links box). Epidemiological studies have shown marked variability in the frequency of occurence of these alleles. The incidence of NAT2 slow acetylators might vary from 5 to 95%, depending on the population that is being studied[24]

Drug reactions, enzymes and genetics

In 1957 Arno Motulsky was the first to recognize the significance of the key discoveries of the 1950s. His seminal paper — *Drug reac-*



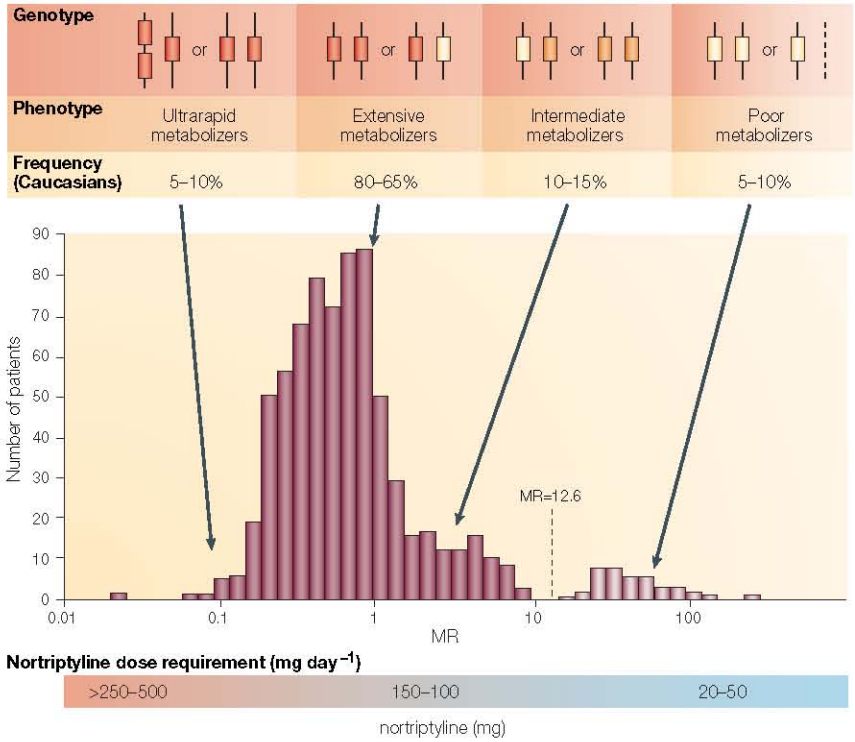


Figure 3 | **Genotype–phenotype relationships of the *CYP2D6*-polymorphism.** Null alleles of the *CYP2D6* gene on chromosome 22 are indicated by yellow boxes, fully functional alleles by red boxes, decreased function alleles by orange boxes, and deletion of the *CYP2D6* gene by a dashed line. The associated phenotypes and their approximate frequencies in Caucasian populations are assigned to the subpopulations that have been determined by the urinary metabolic ratio (MR) of debrisoquine to 4-hydroxy-debrisoquine. MR = 12.6 is the cutoff point between individuals with 'poor metabolism', as a result of decreased or absent CYP2D6 activity, and subjects with intermediate or extensive metabolism. To achieve the same plasma concentration of the antidepressant drug nortriptyline, poor metabolizers require only a fraction of the dose of extensive metabolizers, and ultrarapid metabolizers need a higher dose (modified from REFS 59,60).

tions, enzymes and biochemical genetics — was written on the invitation of a committee of the American Medical Association[12]. Motulsky highlighted the genetic basis of adverse reactions to both primaquine and succinylcholine (see above), but he also mentioned barbiturate-precipitated attacks of acute intermittent porphyria and hereditary HYPERBILIRUBINEMIA. He made the point that these reactions showed "how hereditary gene-controlled enzymatic factors determine why, with identical exposure, certain individuals become 'sick', whereas others are not affected." This paper marked the true beginnings of PGx as a distinct discipline. Recognition of the field spread rapidly. Friedrich Vogel in Heidelberg, Germany, coined the term 'pharmacogenetics' in 1959 (REF. 13). In 1962, Kalow published the first monograph on PGx[14], reviewing all the published examples of genetic factors that influence the response to drugs and chemicals. By 1956 a book with the title *'Biochemical Individuality'* already had a chapter on variability in the responses to drugs[15].

These publications triggered numerous observations over the following years on how genes affect drug responses, and a community of researchers that were interested in this topic started to develop. The first international conference on PGx, held at the New York Academy of Sciences, came in 1967. It brought together workers in many of the newly developing areas of pharmacogenetic research. In addition to G6PD deficiency these areas included MALIGNANT HYPERTHERMIA, FAMILIAL DYSAUTONOMIA, porphyria and red-blood-cell enzymatic defects that are associated with drug-induced haemolysis. Hereditary resistance to coumarin anticoagulants, reduced activity of alcohol dehydrogenase in the liver, adverse drug responses to acetophenetidin (METHEMOGLOBINEMIA and haemolysis) and genetic aspects of allergic reactions to drugs were other topics presented at that conference. They exemplify the wide spectrum and rising awareness of gene–drug interactions.

Regular symposia had a significant role in making PGx known to other disciplines, and many more pharmacogenetic conditions were observed in the following years. For example, starting in 1968, Vessell and co-workers used a series of identical and fraternal twins to assess the genetic contribution to the rate of disappearance of numerous drugs from plasma[16]. Alexanderson *et al.*[17] also used this approach to show the genetic control of steady-state plasma levels of NORTRIPTYLINE. These twin studies provided the most convincing evidence that, for many drugs, genetic factors underlie most of the variability in the rates of metabolism among different individuals. The modern statistical and genetic technologies to analyse such polygenic traits were not available at that time.

In parallel with the realization of the scope of pharmacological variation, more than 100 further examples of exaggerated responses to drugs, novel drug effects, or lack of effectiveness of drugs as a manifestation of inherited individual traits were documented in the 1980s (for reviews, see REFS 18–21). These examples also included a number of genetic disorders or enzymatic defects that predispose their otherwise healthy carriers to an abnormal, absent or adverse drug reaction, for example, WARFARIN resistance. Classic 'pharmacogenetic diseases' include inducible hepatic porphyrias and malignant hyperthermia. These disorders are uncovered or dramatically precipitated by the administration of drugs. Most of these inborn errors are rare, with the exception of G6PD deficiency.

Ecogenetics and toxicogenetics

Genetic variation also influences responses to any kind of environmental or xenobiotic impact (ecogenetics). Probably the first to point out the importance of ecogenetics was Brewer[22]. Ecogenetics is concerned with the dynamic interactions between an individual's genotype and environmental agents or toxicants such as industrial chemicals, pollutants, plant and food components, pesticides, and other chemicals. Examples of ecogenetic variation include differences between individuals in ethanol sensitivity, which is related to acetaldehyde-dehydrogenase deficiency; development of pulmonary emphysema in individuals with α_1-antitrypsin deficiency; and differences in nicotine metabolism that are associated with altered smoking behaviour[20,23]. The related field of toxicogenetics examines an individual's predisposition to carcinogenic, teratogenic, and other toxic effects of drugs and chemicals. Obviously, the principal concepts of PGx and ecogenetics are indistinguishable. The same applies to human variation in response to food components, for example, milk intolerance because of lactase deficiency.

Drug-metabolizing enzymes

Although the early landmarks of pharmacogenetic research concerned several relatively common deficiencies of drug-metabolizing enzymes such as *N*-acetyltransferase-2 (BOX 1) and pseudocholinesterase, the field was clearly invigorated by the discovery of the debrisoquine/sparteine polymorphism of drug oxidation in the 1970s. Two groups independently observed unexpected adverse reactions to these drugs in volunteers that were participating in PHARMACOKINETIC studies. Subsequent studies showed that both drugs are metabolized by the same enzyme, a cytochrome P450 monooxygenase that was later designated as CYP2D6. This enzyme affects the metabolism of numerous other drugs, including antidepressants, ANTI-ARRHYTHMICS and OPIOIDS (reviewed in REFS 24,25). The sometimes clinically dramatic manifestations and the unusual molecular genetics have made the debrisoquine/sparteine polymorphism one of the most well-studied pharmacogenetic traits, as reflected in over 2,500 publications since its discovery.

The CYP2D6 paradigm. In 1975, at St. Mary's Hospital Medical School in London, Robert L. Smith, the laboratory director, ingested 32 mg of debrisoquine, a SYMPATHICOLYTIC antihypertensive drug, as did some of his co-workers. His later account of his adverse response to the drug states: "Within two hours severe orthostatic hypotension set in with blood pressure dropping to 70/50 mm Hg, hypotensive symptoms persisted for up to two days after the dose..."[26]. His colleagues who had ingested a similar dose had no significant cardiovascular effects. Analysis of 4-hydroxydebrisoquine in the urine of the volunteers revealed that the extreme sensitivity was associated with the inability to form this metabolite. A later study of 94 medical students and 3 families who were given a dose of 10 mg of debrisoquine led to the description of this genetic polymorphism of drug oxidation with two phenotypes, the 'poor' and the 'extensive' metabolizers[27].

At the same time, in Bonn, Michel Eichelbaum and Hans Dengler were carrying out a routine pharmacokinetic study to characterize the kinetics of sparteine, an anti-arrhythmic and OXYTOXIC drug. Two individuals in the study had unpleasant side effects such as nausea, DIPLOPIA, blurred vision and headaches — symptoms that are typical of intoxication with this alkaloid. These adverse effects were associated with high plasma levels of sparteine. Subsequent population and family studies showed that sparteine metabolism was subject to a genetic polymorphism that results in two different phenotypes[28].

Both for debrisoquine and for sparteine, the metabolic defect was inherited in a Mendelian (monogenic) fashion as an autosomal-recessive trait. A few years later, the two independent observations converged when it became clear that they were the consequence of the same genetic metabolic deficiency.

The molecular mechanism of the debrisoquine/sparteine polymorphism was discovered a few years later. Biochemical studies in human liver microsomes first indicated that a deficiency of a specific cytochrome-P450 enzyme was responsible[29,30]. This enzyme was purified from human liver in the laboratory of F. P. Guengerich[31] and my own laboratory[32]. Uli Zanger in my laboratory next showed the absence of this protein in the livers of poor metabolizers of debrisoquine (or bufuralol)[33]. In the same year, in a collaboration with the laboratory of Frank Gonzalez at the National Cancer Institute/National Institutes of Health, the cDNA of this cytochrome-P450 enzyme, which was now called CYP2D6, was cloned[34]. This cDNA was used to show the inheritance of several defective alleles of *CYP2D6* by restriction-fragment length polymorphism (RFLP) analysis in several families of poor debrisoquine metabolizers[35]. A detailed sequence analysis of the two most frequent alleles (*CYP2D6*4* and *CYP2D6*3*) and the functional importance of each mutation was analyzed in heterologous expression experiments[36,37]. The timely emergence of PCR technology also allowed us to develop the first allele-specific PCR-test to identify the most common genotypes of poor metabolizers of debrisoquine[38].

Clinical observations and phenotyping of patients with debrisoquine 'urinary metabolic ratios' (FIG 3) in Sweden led to another interesting observations in 1993. Previously, Bertilsson *et al.*[39] had described an extremely high oxidation capacity for nortriptyline in a woman who was resistant to the normal doses of this antidepressant. Inger Johansson in Magnus Ingelman-Sundberg's laboratory detected unusual RFLP patterns in the DNA of the 'ultrarapid' metabolizers and found up to 12 extra copies of the *CYP2D6* gene fused in a head-to-tail orientation on chromosome 22 in these subjects[40]. Retrospective analysis of the DNA from the non-responder to antidepressant therapy also revealed that this patient was the carrier of three copies of *CYP2D6*[41]. This represents the first description of a stably amplified, functionally active, human gene, in which the amplification is inherited as dominant trait. However, not all of the ultrarapid metabolizers can be explained by gene duplication/amplification.

Since these discoveries, numerous additional *CYP2D6* alleles were discovered and their frequencies and functional significance were studied worldwide. The Home Page of the *CYP* allele nomenclature committee (see further information in the online links box) currently lists 44 alleles, including gene deletions and duplications, with a total number of 78 distinct variants. A number of these variants can be associated with four metabolism phenotypes: poor, intermediate, extensive and ultrarapid (FIG. 3).

The large number of important drug substrates for CYP2D6 has stimulated numerous studies of the genotype–phenotype rela-

Box 2 | Therapeutic lessons from pharmacogenetics

- All drug effects vary from person to person and all drug effects are influenced by genes.
- Most drug responses are multifactorial (that is, many genes and many environmental factors contribute to them).
- Genetic polymorphisms of single genes, including mutations in coding sequences, gene duplications, gene deletions and regulatory mutations affect numerous drug-metabolizing enzymes. Several cytochrome-P450 enzymes (for example, CYP2D6 and CYP2C9), *N*-acetyltransferases (NAT2), thiopurine methyltransferase (TPMT) and UDP-glucuronosyltransferases (UDP-GT) are examples. Individuals that possess these polymorphisms are at risk of experiencing documented adverse reactions or inefficacy of drugs at usual doses.
- Genetic polymorphisms of drug targets and drug transporters are increasingly recognized (receptors, ion channels, growth factors) as causing variation in drug responses.
- Several targets of cancer therapy, for example, the epidermal-growth-factor receptor, respond to treatment only in subgroups of patients who carry sensitizing mutations of these targets[48,49].
- The frequency of variation of drug effects, whether multifactorial or genetic, varies considerably in ethnically defined populations (for example, alleles of *N*-acetyltransferases)[24].
- Application of response-predictive genetic profiles (for example, genotyping for polymorphisms in antidepressant or cancer-drug therapy) on clinical outcomes has, so far, been done mostly in academic centers and has not yet reached clinical practice.

tionship of this polymorphism, as well as studies of disease associations and inter-ethnic variability. CYP2D6 seems to be the most variable and most well-investigated member of the cytochrome-P450 superfamily of genes.

Other drug-metabolizing enzymes. In the years after the discovery of the *CYP2D6* polymorphism, other polymorphisms of cytochrome-P450 genes were discovered, notably the MEPHENYTOIN polymorphism[42], which is due to a deficiency of CYP2C19 (REF. 43) and leads to an enhanced effect of the antiulcer drug omeprazole. The phenytoin/warfarin polymorphism, which is caused by mutations of *CYP2C9* (REF. 44), is another good example. This polymorphism affects the metabolism of the anticoagulant warfarin and the anticonvulsant phenytoin (Dilantin; Pfizer Inc.), as well as some other drugs.

In the decade that followed the elucidation of the mechanism of the *CYP2D6* polymorphism, many other genes that were responsible for such genetic polymorphisms were identified, functionally characterized, and linked to inherited differences in drug effects. One important example is the polymorphism of thiopurine *S*-methyltransferase (TPMT), with its clinically dramatic influence on the toxicity of the anticancer and immunosuppressive agents mercaptopurine and azathioprine (Imuran; GlaxoSmithKline). Other examples are the polymorphism of UDP-glucuronosyltransferase UGT1A1 and its effects on metabolism and toxicity of the anticancer drug irinotecan, and the deficiency of dihydropyrimidine dehydrogenase, which causes increased toxicity of the anticancer drug fluorouracil for a review of these topics, see REF. 45). Genotyping tests for *CYP2D6*, *TPMT*, *CYP2C9* and *UGT1A1* have been recommended to help make treatment decisions, but the routine use of genotyping is still in its infancy.

Transporters and drug targets. During the last 10 years, a number of polymorphisms of genes that encode drug transporters and drug targets have also been discovered and shown to alter drug responses (for review, see REFS 46,47). Of particular interest are the recent reports of the effectiveness of a generally ineffective drug, gefitinib (Iressa; AstraZeneca) in lung cancer patents that carry a sensitizing mutation of the epidermal growth factor receptor[48,49]. Such observations highlight the many sources of genetic variation that influence drug responses.

The challenge of multifactorial drug responses. At this time, the well-established examples of PGx with clinical relevance are pharmacogenetic diseases and genetic polymorphisms that alter the metabolism of drugs (BOX 2). All these are monogenic traits with Mendelian inheritance. However, most drug effects and treatment outcomes, or the individual risk for drug inefficacy or toxicity, are due to complex interactions between genes and the environment. Environmental variables include nutritional factors, concomitantly administered drugs, disease, and many other factors including lifestyle influences such as smoking and alcohol consumption. These factors work together with several individual genes that code for pharmacokinetic and PHARMACODYNAMIC determinants of drug effects such as receptors, ion channels, drug-metabolizing enzymes and drug-transporters. The challenge will be to define polygenic determinants of drug effects and to use a combination of genotyping and phenotyping tests to assess environmental influences[50]

Glossary

ALCAPTONURIA
A rare inherited disorder of metabolism that is characterized by urine which turns black when exposed to air.

ANTI-ARRHYTHMICS
Medicines that are used to treat patients who have irregular heart rhythms.

APNEA
The absence of breathing (respirations).

CHEMICAL INDIVIDUALITY
Garrod's influential idea that 'factors which confer upon us our predisposition and immunities from disease are inherent in our very chemical structure, and even in the molecular groupings which went to the making of the chromosomes from which we sprang.'

DIPLOPIA
Double vision. Usually due to misalignment of the eyes.

FAMILIAL DYSAUTONOMIA
A disorder of the autonomic nervous system that is inherited as an autosomal recessive trait and is characterized by several sensory deficits (as of taste and pain), excessive sweating and salivation, lack of tears, difficulty in swallowing and many other symptoms.

HYPERBILIRUBINEMIA
Abnormally high levels of bilirubin in the blood.

ISONIAZID
An anti-bacterial drug that has been used to prevent and to treat tuberculosis since 1952.

MALIGNANT HYPERTHERMIA
A group of inherited muscle problems characterized by muscle breakdown following certain stimuli — such as anesthesia, extremes of exercise (particularly in hot conditions), fever, or use of stimulant drugs. The problems associated with this condition result from over-excitable muscles that contract uncontrollably, severe fever, abnormal heart rhythms, and kidney failure.

MEPHENYTOIN
An anticonvulsant that is indicated for the treatment of tonic-donic and partial seizures in patients who are not controlled with less-toxic medications.

METHEMOGLOBINEMIA
An inherited blood disorder that is characterized by increased levels of an abnormal form of haemoglobin that is unable to deliver oxygen effectively.

NORTRIPTYLINE
An antidepressant medication of the tricyclic class. Medications in this class are often referred to as tricyclic antidepressants, or TCAs.

OPIOIDS
Synthetic opium-like drugs that possess some affinity for any, or all, of the opioid-receptor subtypes. Common opioids are endorphin, fentanyl and methadone.

OXYTOXIC
A drug that is useful in starting or aiding in labour. Also used to stimulate uterine contractions.

PERIPHERAL NEUROPATHY
A problem in peripheral nerve function (any part of the nervous system except the brain and spinal cord) that causes pain, numbness, tingling, swelling, and muscle weakness in various parts of the body. Neuropathies might be caused by physical injury, infection, toxic substances, disease (for example, cancer, diabetes, kidney failure, or malnutrition), or drugs such as anticancer drugs.

PHARMACODYNAMICS
The process of interaction of pharmacologically active substances with target sites, and the biochemical and physiological consequences leading to therapeutic or adverse effects.

PHARMACOKINETICS
The rocess of the uptake of drugs by the body, the biotransformation they undergo, the distribution of the drugs and their metabolites in the tissues, and the elimination of the drugs and their metabolites from the body.

PORPHYRIA
A group of disorders that are characterized by the excessive production of porphyrins or their precursors, and which arise from abnormalities in the regulation of the porphyrin–heme pathway. Acquired pophyrias, which are due to inhibition of enzymes in the metabolic pathway by a drug, toxin or abnormal metabolite, are more common than those that are inherited.

PSEUDOCHOLINESTERASE DEFICIENCY
A rare genetic disorder that causes an absence of the plasma enzyme pseudocholinesterase, which can cause respiratory difficulty during surgery if the muscle-relaxing drug succinylcholine is used.

SYMPATHICOLYTIC
Interfering with, opposing, inhibiting, or destroying impulses from the sympathetic nervous system.

WARFARIN
An oral anticoagulant that inhibits the synthesis of clotting factors, thus preventing blood-clot formation.

Pharmacogenomics

The increasing use of the term pharmacogenomics (FIG. 1) reflects the evolution of PGx into the study of the entire spectrum of genes that determine drug responses, including the assessment of the diversity of the human genome sequence and its clinical consequences. There are three aspects of pharmacogenomics that make it different from classic PGx.

Genetic drug-response profiles. Rapid sequencing and genotyping of SNPs will have a significant role in associating sequence variations with heritable clinical phenotypes of drug or xenobiotic responses. SNPs occur approximately once every 300–3,000 bp if the genomes of 2 unrelated individuals are compared, and these represent 90–95% of all variant DNA sites. Any two individuals therefore differ at approximately 3–10 million bp, that is, <1% of the 3.2 billion bp of the haploid genome.

How can we use this information to predict drug responses? Pharmacogenomics focuses on SNPs for the simple and practical reason that they are both the most common and the most technically accessible class of genetic variations. For clinical-correlation studies in relatively small populations SNPs that occur at frequencies >10% are most likely to be useful, but rare SNPs with a strong selection component and a more marked effect on phenotype are equally important. Once a large number of these SNPs and their frequencies in different populations are known, they can be used to correlate an individual's genetic 'fingerprint' with their probable drug response. It has been proposed that high-density maps of SNPs or so-called haplotype blocks (sets of SNPs that are inherited together; see the International HapMap Project in the online links box) in the human genome might allow the use of these SNPs as markers of xenobiotic responses even if the target remains unknown, providing a 'drug-response profile' that is associated with contributions from many genes to a response phenotype. However, SNPs are not evenly distributed across the genome and differ between populations of different ethnic origin. In practice, therefore, and because of the complexities of defining disease phenotypes and clinical outcomes, the validity of this concept remains to be shown. Obviously, phenotyping methods will remain extremely important to assess the clinical relevance of genetic variations, as discussed below.

The effect of drugs on gene expression. Genomic technologies also include methods to study the expression of large groups of genes and indeed the entire complement of products (mRNAs) of a genome. Most drug actions produce changes in gene expression in individual cells or organs. This provides a new perspective for the way in which drugs interact with the organism and provides a measure of the biological effects of the drug. For instance, numerous drugs induce their own metabolism and the metabolism of other drugs by interacting with nuclear receptors such as AhR (arylhydrocarbon receptor), PPAR (peroxisome proliferator activated receptor), PXR (pregnane X receptor) and CAR (constitutive androstane receptor). These receptors function as 'xenosensors' and transcription factors that activate a response that includes increased biotransformation of drugs (reviewed in REF. 51). The phenomenon of induction has significant clinical consequences such as altered kinetics, drug–drug interactions or changes in hormone and carcinogen metabolism. Genomics is providing the technology to better analyze these complex multifactorial situations and to obtain individual genotypic and gene-expression information to assess the relative contributions of environmental and genetic factors to variations in drug responses.

Pharmacogenomics in drug discovery and drug development. The identification of all genes and, ultimately, the study of all protein variants that cause, contribute to, or modify a disease, will lead to new 'drugable' and 'nondrugable' targets, prognostic markers of disease states or severity-of-disease information. The pharmaceutical industry has realized this potential. It is obvious that the discovery of genes and proteins that are involved in the pathogenesis of disease allows the definition of new drug targets and promises to change profoundly the field of medicine in the future.

The promise of personalized medicine. Is the promise that pharmacogenomics will provide more 'personalized medicine' a reasonable expectation? And if so, why is PGx so rarely applied in clinical practice, in spite of well-established genetic polymorphisms and available genotyping methods? Numerous reasons for the slow acceptance of pharmacogenetic principles have been brought forward[46,50]. The lack of large prospective studies to evaluate the impact of genetic variation on drug therapy is one reason for the slow acceptance of these principles. On the other hand, pharmacogenetic information is increasingly included in product information or drug data sheets that alert the physician to dosing problems. Recent retrospective analysis of psychiatric patients that were treated with drugs that are substrates of CYP2D6 strongly indicates that genotyping can improve efficacy, prevent adverse drug reactions, and lower the costs of therapy with these agents[52]. The future impact of PGx and pharmacogenomics is likely to be considerable both in the selection of the right drug at the proper 'individual' dose and in the prevention of adverse effects (FIG. 3). By translating the increasing knowledge of human genetic diversity into better drug treatment, improved health through personalized therapy remains a realistic future scenario in many fields of medicine.

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1. Garrod, A. E. The incidence of alkaptonuria. A study in chemical individuality. *Lancet*, 1616–1620 (1902).
2. Garrod, A. E. *The inborn errors of metabolism*, (Oxford Univ. Press, London, UK, 1909).
3. Bateson, W. *Mendel's principles of heredity: A defence*, (Cambridge Univ. Press, Cambridge, UK, 1902).
4. Garrod, A. E. *The inborn factors of disease* (Oxford Univ. Press, London, UK, 1931).
5. Fox, A. L. The relationship between chemical constitution and taste. *Proc. Natl Acad. Sci. USA* **18**, 115–120 (1932).
6. Snyder, L. H. Studies in human inheritance IX. The inheritance of taste deficiency in man. *Ohio J. Sci.* **32**, 436–468 (1932).
7. Clayman, C. B. *et al.* Toxicity of primaquine in Caucasians. *J. Am. Med. Assoc.* **149**, 1563–1568 (1952).
8. Carson, P. E., Flanagan, C. L., Ickes, C. E. & Alving, A. S. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* **124**, 484–485 (1956).
9. Luzatto, L., Mehta, A. & Vulliamy, M. Y. in *The Metabolic and Molecular Basis of Inherited Diseases* Vol. 3 (eds Scriver, C. R. *et al.*) 4517–4553 (McGraw–Hill Inc., New York, USA, 2001).
10. Lehmann, H. & Ryan, E. The familial incidence of low pseudocholinesterase level. *Lancet* **271**, 124 (1956).
11. Kalow, W. & Staron, N. On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. *Can. J. Med. Sci.* **35**, 1305–1320 (1957).
12. Motulsky, A. Drug reactions, enzymes and biochemical genetics. *JAMA* **165**, 835–837 (1957).
13. Vogel, F. Moderne Probleme der Humangenetik. *Ergebn. Inn. Med. Kinderheilkd.* **12**, 52–125 (1959).
14. Kalow, W. *Pharmacogenetics: heredity and the response to drugs* (W. B. Saunders & Co., Philadelphia, USA, 1962).
15. Williams, R. J. *Biochemical Individuality*, (John Wiley & Sons, New York, USA, 1956).
16. Vesell, E. S. Twin studies in pharmacogenetics. *Hum. Genet.* **1**, (Suppl.) 19–30 (1978).
17. Alexanderson, B., Evans, D. A. & Sjoqvist, F. Steady-state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy. *Br. Med. J.* **4**, 764–768 (1969).
18. Price-Evans, D. A. in *Clinical and Molecular Pharmacogenetics* (Cambridge Univ. Press, Cambridge, UK, 1993).
19. Evans, W. E. & McLeod, H. L. Pharmacogenomics — drug disposition, drug targets, and side effects. *N. Engl. J. Med.* **348**, 538–549 (2003).
20. Weber, W. W. *Pharmacogenetics* 334 (Oxford Univ. Press, Oxford, UK, 1997).
21. Kalow, W., Meyer, U. A. & Tyndale, R. F. *Pharmacogenomics* (Marcel Dekker, New York and Basel, 2001).
22. Brewer, G. J. Annotation: human ecology, an expanding role for the human geneticist. *Am. J. Hum. Genet.* **23**, 92–94 (1971).
23. Sellers, E. M., Tyndale, R. F. & Fernandes, L. C. Decreasing smoking behaviour and risk through CYP2A6 inhibition. *Drug Discov. Today* **8**, 487–493 (2003).
24. Meyer, U. A. & Zanger, U. M. Molecular mechanisms of genetic polymorphisms of drug metabolism. *Annu. Rev. Pharmacol. Toxicol.* **37**, 269–296 (1997).
25. Zanger, U. M., Raimundo, S. & Eichelbaum, M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch. Pharmacol.* **369**, 23–37 (2004).

26. Smith, R. L. Introduction: human genetic variations in oxidative drug metabolism. *Xenobiotica* **16**, 361–365 (1986).
27. Mahgoub, A., Idle, J. R., Dring, L. G., Lancester, R. & Smith, R. L. Polymorphic hydroxylation of debrisoquine in man. *Lancet* **2**, 584–586 (1977).
28. Eichelbaum, M., Spannbrucker, N., Steincke, B. & Dengler, H. J. Defective *N*-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur. J. Clin. Pharmacol.* **16**, 183–187 (1979).
29. Kahn, G. C., Boobis, A. R., Murray, S., Brodie, M. J. & Davies, D. S. Assay and characterisation of debrisoquine 4-hydroxylase activity of microsomal fractions of human liver. *Br. J. Clin. Pharmacol.* **13**, 637–645 (1982).
30. Meier, P. J., Mueller, H. K., Dick, B. & Meyer, U. A. Hepatic monooxygenase activities in subjects with a genetic defect in drug oxidation. *Gastroenterology* **85**, 682–692 (1983).
31. Distlerath, L. M. *et al.* Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine
4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.* **260**, 9057–9067 (1985).
32. Gut, J. *et al.* Debrisoquine-type polymorphism of drug oxidation: purification from human liver of a cytochrome P450 isozyme with high activity for bufuralol hydroxylation. *FEBS Lett.* **173**, 287–290 (1984).
33. Zanger, U. M., Hauri, H. P., Loeper, J., Homberg, J. C. & Meyer, U. A. Antibodies against human cytochrome P-450db1 in autoimmune hepatitis type II. *Proc. Natl Acad. Sci. USA* **85**, 8256–8260 (1988).
34. Gonzalez, F. J. *et al.* Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* **331**, 442–446 (1988).
35. Skoda, R. C., Gonzalez, F. J., Demierre, A. & Meyer, U. A. Two mutant alleles of the human cytochrome P-450db1 gene (*P450C2D1*) associated with genetically deficient metabolism of debrisoquine and other drugs. *Proc. Natl Acad. Sci. USA* **85**, 5240–5243 (1988).
36. Kagimoto, M., Heim, M., Kagimoto, K., Zeugin, T. & Meyer, U. A. Multiple mutations of the human cytochrome P450IID6 gene (*CYP2D6*) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes.
J. Biol. Chem. **265**, 17209–17214 (1990).
37. Gough, A. C. *et al.* Identification of the primary gene defect at the cytochrome P450 *CYP2D* locus. *Nature* **347**, 773–776 (1990).
38. Heim, M. & Meyer, U. A. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* **336**, 529–532 (1990).
39. Bertilsson, L., Aberg-Wistedt, A. & Gustaffson, L. L. Extremely rapid hydroxylation of debrisoquine; a case report with implication for treatment with nortriptyline and other tricyclic antidepressants. *Ther. Drug. Monit.* **7**, 478–480 (1985).
40. Johansson, I. *et al.* Inherited amplification of an active gene in the cytochrome P450 *CYP2D* locus as a cause of ultrarapid metabolism of debrisoquine. *Proc. Natl Acad. Sci. USA* **90**, 11825–11829 (1993).
41. Bertilsson, L. *et al.* Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. *Lancet* **341**, 63 (1993).
42. Küpfer, A. & Preisig, R. Pharmacogenetics of mephenytoin: a new drug hydroxylation polymorphism in man. *Eur. J. Clin. Pharmacol.* **26**, 753–759 (1984).
43. de Morais, S. M. F. *et al.* Identification of a new genetic defect responsible for the polymorphism of *S*-mephenytoin metabolism in Japanese. *Mol. Pharmacol.* **46**, 594–598 (1994).
44. Sullivan-Klose, T. H. *et al.* The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* **6**, 341–349 (1996).
45. Weinshilboum, R. Inheritance and drug response. *N. Engl. J. Med.* **348**, 529–537 (2003).
46. Goldstein, D. B., Tate, S. K. & Sisodiya, S. M. Pharmacogenetics goes genomic. *Nature Rev. Genet.* **4**, 937–947 (2003).
47. Roden, D. M. & George, A. L. Jr. The genetic basis of variability in drug responses. *Nature Rev. Drug Discov.* **1**, 37–44 (2002).
48. Lynch, T. J. *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**, 2129–2139 (2004).
49. Paez, J. G. *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
50. Nebert, D. W., Jorge-Nebert, L. & Vesell, E. S. Pharmacogenomics and 'individualized drug therapy': high expectations and disappointing achievements. *Am. J. Pharmacogenomics* **3**, 361–370 (2003).
51. Handschin, C. & Meyer, U. A. Induction of drug metabolism: the role of nuclear receptors. *Pharmacol. Rev.* **55**, 649–673 (2003).
52. Chou, W. H. *et al.* Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. *J. Clin. Psychopharmacol.* **20**, 246–251 (2000).
53. Bönicke, R. & Reif, W. Enzymatische Inaktivierung von Isonicotinsäure hydrazide im menschlichen und tierischen Organismus. *Arch. Exp. Pathol. Pharmakol.* **220**, 321–333 (1953).
54. Hughes, H. B., Biehl, J. P., Jones, A. P. & Schmidt, L. H. On the metabolic fate of isoniazid. *J. Pharmacol. Exp. Therap.* **109**, 444–452 (1953).
55. Hughes, H. B., Biehl, J. P., Jones, A. P. & Schmidt, L. H. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am. Rev. Tuberc.* **70**, 266–273 (1954).
56. Evans, D. A. P., Manley, F. A. & McKusick, V. A. Genetic control of isoniazid metabolism in man. *Br. Med. J.* **2**, 485–491 (1960).
57. Blum, M., Demierre, A., Grant, D. M., Heim, M. & Meyer, U. A. Molecular mechanism of slow acetylation in man. *Proc. Natl Acad. Sci. USA.* **88**, 5237–5241 (1991).
58. Vatsis, K. P., Martel, K. J. & Weber, W. W. Diverse point mutations in the human gene for polymorphic *N*-acetyltransferase. *Proc. Natl Acad. Sci. USA* **88**, 6333–6337 (1991).
59. Meyer, U. A. Pharmacogenetics and adverse drug reactions. *Lancet* **356**, 1667–1671 (2000).
60. Kirchheiner, J. *et al.* Individualized medicine — implementation of pharmacogenetic diagnostics in antidepressant drug treatment of major depressive disorders. *Pharmacopsychiatry* **36** (Suppl. 3), S235–S243 (2003).
61. Vatsis, K. P. *et al.* Nomenclature for *N*-acetyltransferases. *Pharmacogenetics* **5**, 1–17 (1995).
62. Masood, E. A. A consortium plans free SNP map of human genome. *Nature* **398**, 545–546 (1999).
63. Scriver, C. R. & Childs, B. (eds) *Garrod's Inborn Factors Of Disease* (Clarendon Press, Oxford, UK, 1989).

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Competing interests statement
The author declares no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
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AhR | CAR | *CYP2C9* | CYP2C19 | CYP2D6 | G6PD | *NAT2* | TPMT | UGT1A1
Home Page of the *CYP* Allele Nomenclature Committee: http://www.imm.ki.se/CYPalleles/
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VIEWPOINT

Pharmacogenetics: ethical problems and solutions

Alasdair Breckenridge, Klaus Lindpaintner, Peter Lipton, Howard McLeod, Mark Rothstein and Helen Wallace

Abstract | Regulators, drug companies, academic scientists, bioethicists, clinicians and, increasingly, the general public are starting to realize that pharmacogenetics (PGx) will probably have a huge impact on the way in which we treat both common and rare diseases. But how much thought has gone into the ethical issues that the incorportation of phamacogenetic testing into drug discovery, prescription and use will entail? It seems that "quite a bit" is the answer, as the diverse viewpoints of representatives of all of these groups presented here illustrate. However, these views also highlight that now is the time to start formulating and implementing solutions to these potential problems.

What do you see as the most important future benefits of PGx to society and, conversely, what are the main problems that it poses?

• Individuals differ in their response to medicines for many reasons, one of the most important being differences in their genetic make-up. PGx is the study of genetic variations that affect responses to medicines, both in terms of efficacy and safety. For certain medicines, appropriate genetic testing could, in theory, select those patients who are likely to derive more benefit from a medicine or, conversely, those who are more likely to suffer an adverse effect. This has implications not only for individual treatment, but also for the way in which medicines are developed. Clinical trials of new medicines conducted in cohorts of patients selected by PGx testing could be smaller and could be carried out more quickly, which would result in lower development costs. Evaluation of the safety profile of medicines that are already on the market is also an important area in which PGx profiling can be used.

Much is made of how this approach raises concerns about patient confidentiality and