Pharmacogenomics steps toward personalized medicine

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The goal of personalized medicine is to maximize the likelihood of therapeutic efficacy and to minimize the risk of drug toxicity for an individual patient. One of the major contributors to this concept is pharmacogenomics. Marked interindividual genetic variation contributes significantly to both susceptibility to diseases, and response to drugs. Even though pharmacogenomics is not a new science, the translation of pharmacogenomics into clinical practice (i.e., personalized medicine) has not taken place at the same pace as science is delivering new results. It is felt that a large number of recent pharmacogenomic findings allow bold steps to be taken toward personalized medicine. This review collates a variety of examples that have great potential for immediate and effective introduction into clinical practice. In addition, other exploratory examples with a particular focus on drug safety and targeted cancer therapy are summarized.

Keywords: customized medication, gene–drug interaction, genetic personalization, individualized treatment, mechanism-based therapeutics, personalized medicine, pharmacogenetics, pharmacogenomics, tailored dosage, targeted therapy

future medicine

During the past decade, in particular after the completion of the Human Genome Project [1,2], an explosion of information regarding genetic susceptibility to complex diseases and genetic variability in drug responses was observed. Genomics has become an integral part of modern drug development, and a large number of pharmaceutical companies are using this information to identify novel drug targets, identify patient subpopulations that are likely to benefit from the therapy under development, or for other screening purposes. While this new knowledge has led to concrete clinical advances, it has also led to speculation about the projected benefit from pharmacogenomics for the individual patient: what has been missing was the effective translation of this information into clinical practice. Even though several reasons exist as to why this hasn't happened yet, one major issue is the lack of provision of effective information regarding the benefit of pharmacogenomics to physicians and patients. Should true personalized medicine become the reality that is hoped for, pharmacogenomics needs to become fully integrated into the strategy of identifying the best possible drug therapies for the prevention, diagnosis and treatment of disease. However, a genetic or genomic profile alone will, only in rare occasions, be a definite determinant of therapy: other factors such as age, gender, body mass, or potential drug–drug interactions need to be considered as well, leaving the burden of making an educated, clinical decision about treatment in the hands of physicians. However, pharmacogenomics, the science of how an individual's genetic makeup influences a person's reaction to drugs, can greatly improve this decision-making process by providing critical insights into how this patient will react to the treatment of choice.

Current status of drug therapy

The safety and efficacy of a drug is evaluated according to strict regulatory guidelines before the drug is marketed. However, it is impossible for an approved drug to be safe or effective for everyone. Genetic and environmental factors, including their interactions, result in substantial variability among individuals. Environmental factors (also called nongenetic factors) include not only true environmental factors, such as diet and drug–drug interactions, but also others such as age and disease. The genetic makeup of every individual is unique, resulting in significant variability in the expression of gene products such as drug-metabolizing enzymes, drug transporters, drug targets and their downstream signal transduction molecules. For example, there is up to a 20-fold difference in the dosages of warfarin required to achieve the desired therapeutic effect when given to different patients [3–5], and the plasma concentrations of a drug vary 30- to 50-fold among individuals receiving the same dose [6]. Variability in clinical drug response is supported by a recent survey showing that efficacy rates for drug therapy of most diseases fall in the range of 25–80% (mostly 50–75%) and that 20% or more of patients being treated are expected to be poor responders [7]. The traditional standard approaches to drug development and clinical therapies, such as 'trial and error',

'one drug fits all', and 'one dose fits all', are very limited, contributing to 25–50% of drug toxicity or treatment failures [7].

Drug safety varies from drug to drug, from person to person, and even from disease to disease. For drugs that have been approved in the USA, 16% (193 out of 1232) have been found to be associated with severe adverse drug reactions [7], and 27 drugs are frequently cited as causes of adverse drug reaction studies [8]. In an effort to explore possible causes, a survey in which 2227 cases of adverse drug events were investigated among hospitalized patients, found that 50% of these cases were likely to be associated with genetic factors [9]. Of the above often-cited 27 drugs, 59% are metabolized by at least one enzyme known to have allelic variants that cause poor metabolism [8]. The clinical and economic impact of these adverse events has been described in various reports [10–15]. Among the most cited evidence are the following findings:

- At least 6% of new hospital admissions to internal medicine wards are due to various serious adverse drug reactions in the developed countries [10–12]
- A frequently cited meta-analysis showed that an estimated 2 million people were hospitalized because of serious adverse drug events in the USA, with the number of deaths exceeding 100,000 cases annually, representing the fifth leading cause of death [13]
- The costs of drug-related morbidity and mortality are expected to exceed US$177 billion annually in the USA [14]
- Many drugs have been withdrawn from the major markets only because they cause severe toxicity in a small number of people [15]

Genetic basis of variable drug response

The phenotype of drug response is highly complex, representing a classic example of the outcome of gene–drug interactions. Clinically important variable drug responses at usual doses are categorized into four classes: inefficacy, efficacy, resistance, and toxicity. Of the drug-metabolizing enzymes, three clinically important isoforms – cytochrome P450 (CYP)2D6, CYP2C9, and CYP2C19 (which contribute to the metabolism of 40% of the overall marketed drugs) – possess a large number of functionally significant genetic polymorphisms [201]. For drugs that have a narrow therapeutic index and are inactivated by a certain polymorphic drug-metabolizing enzyme, poor metabolizers (PMs) are expected to have an increased risk of adverse drug reactions, whereas ultrarapid metabolizers (UMs) have reduced drug effects. For drugs that require a metabolic activation by a polymorphic drug-metabolizing enzyme, the so-called prodrugs (for example, codeine), PMs have a reduced efficacy or treatment failure, whereas UMs have significantly increased efficacy, and even toxicity. For many drugs that have a broad therapeutic window, individuals carrying a gene variant or variants may exhibit impaired drug metabolism and disposition, but these may be of limited clinical relevance. The number of examples relating to this field is growing rapidly [15–24]. Some examples of drugs that have great potential for personalization have been summarized elsewhere [3,4,6–8,15–24], and other relatively new examples are discussed in this review.

Thiopurines

The thiopurine drugs, azathioprine (AZA), 6-mercaptopurine (6-MP), and thioguanine (TG), are widely used in clinical practice, such as for childhood acute lymphoblastic leukemia (ALL), organ transplant rejection, and rheumatic diseases. The polymorphic thiopurine *S*-methyltransferase (TPMT) catalyzes the *S*-methylation of 6-MP and AZA (after conversion of AZA to 6-MP), with genetically deficient TPMT leading to increased production of 6-thioguanine nucleotides (6-TGNs) that exert therapeutic and toxic hematologic effects. Thus, of clinical significance is how to balance a lower risk of drug toxicity (such as serious and life-threatening myelosuppression) and a higher risk of therapy failure or disease relapse (such as ALL). A large number of studies have shown that several common defective *TPMT* variant alleles (*TPMT *2*, **3A* and **3C*) result in an impaired ability to metabolize AZA and 6-MP [25]. Since there is a significant correlation between *TPMT* genotype and TPMT activity measured in peripheral erythrocytes, individualization of drug dosing could be improved by determining genotype or, more frequently, phenotype [26]. Compared with the carriers of the *TPMT*1* allele who have normal activity, patients who have intermediate activity, or are heterozygous for the *TPMT*1* allele, require 65% of the conventional dosage, whereas TPMT-deficient patients require 5–10% of above dose. Currently, this genetic polymorphism is widely thought to be one of the best examples of the translation of genomic information into clinical practice. For example, it was

estimated that *TPMT* genetic tests were performed over 400 times throughout Australia and New Zealand in 2003 [27]. Prospective genotyping or phenotyping is able to improve TPMT-associated drug therapy and avoid drug toxicity [28–30]. The US Food & Drug Administration (FDA) has updated the labels of 6-MP and AZA to inform consumers about the risk of toxicity, and recommends genotyping before the initiation of treatment with these drugs.

Oral anticoagulants

The coumarin anticoagulants, including warfarin, acenocoumarol and phenprocoumon, are a class of widely-prescribed oral anticoagulants, each having a narrow therapeutic index and wide interindividual variation in dose requirements. A large number of pharmacogenetic studies have revealed that genetic polymorphisms in the genes encoding drug-metabolizing enzyme CYP2C9 [31], the warfarin target protein vitamin K epoxide reductase complex 1 (VKORC1) [32–34] and vitamin K-dependent proteins [35], such as clotting factors II (prothrombin) and VII, as well as γ-glutamyl carboxylase (GGC), may affect the response to these drugs. For the *CYP2C9* gene, some evidence indicated that *CYP2C9*2* was associated with reduced warfarin dose requirement, whereas all studies demonstrated that individuals carrying at least one *CYP2C9*3* allele required a lower than normal dose for all three coumarin anticoagulants than those without this variant [31,36,37]. More importantly, the carriers of these variants (in particular the *CYP2C9*3* allele) appeared more likely to experience bleeding events due to overanticoagulation after taking conventional doses. This is because the variant enzymes exhibit a markedly impaired ability to catalyze the metabolism of the pharmacologically more active *S*-enantiomers of these drugs, leading to increased plasma drug levels. For the target gene *VKORC1*, ten common polymorphisms were identified in its promoter region and five major haplotypes inferred, in which haplotypes 1 and 2 were shown to be associated with a low-dose requirement of warfarin (collectively designated as haplotype group A), whereas the haplotypes 7–9 (designated haploytpe group B) were responsible for a high-dose requirement [33,34].

Evidence shows that the ranking order of the estimated proportion (R^2) of each component accounting for the overall observed variation in warfarin dosing is 35% for clotting factor VII polymorphisms [35], 21–31% for 3–5 common *VKORC1* haplotypes [4,33,34], 9–22% for patient age [4,34,38–40], 6–14% (median: 10%) for *CYP2C9* genotype [4,31–34], 3–9% for *GGC* gene variation [4,35], 5–6% for the patient body size [4,40], and 1% for clotting factor II [35], with a wide variation, depending on ethnicity. Clearly, an individual patient's genetic factors, including gene–gene and allele–allele interactions [34], are the major determinant of the wide variation in warfarin dose requirements, accounting for approximately 75–85% of the overall variability. In addition, some nongenetic factors, such as patient age, body size (height, weight and surface area), ethnic origin, gender, diet, disease status and concomitant medication are also involved. However, a large-scale, prospective clinical investigation, into which all known factors are integrated, is lacking.

It is important to note that, although the example that warfarin dose requirements may vary by *CYP2C9* genotype is one of the most-often-cited examples of a clinically relevant phenotype/genotype correlation study, the above cited recent results indicate that *CYP2C9* itself cannot account for the overall variation in warfarin dose. This example illustrates that multiple genes are responsible for variable metabolism and response to drugs. Accordingly, individualization of drug dosing should take all known factors into account.

Phenytoin & carbamazepine

Phenytoin and carbamazepine are important first-line antiepileptic drugs, each having a relatively narrow therapeutic index and wide interindividual range of doses required. Accumulating evidence has shown that phenytoin is extensively metabolized in the human liver by CYP2C9 and, to a lesser extent, by CYP2C19, whereas carbamazepine is metabolized by CYP3A4. Finally, both drugs are transported across the blood–brain barrier to bind their therapeutic targets, voltage-sensitive sodium channels in neurons (SCNs), blocking their high-frequency discharges. In earlier case reports, patients homozygous for the variant *CYP2C9*3* [41] or *CYP2C9*6* [42] experienced severe phenytoin toxicity as a result of markedly impaired metabolism after taking a usual dose. In a retrospective cohort study [43], functionally significant *CYP2C9*3* and an intronic, functional polymorphism in the *SCN1A* gene (IVS5–91G allele) were associated with a markedly reduced maximum phenytoin dose required, alone and in combination. Similarly,

the *SCN1A* IVS5–91 G allele was also linked to significantly reduced maximum carbamazepine doses [43], suggesting a functional replication of the effect of the variant *SCN1A* gene.

Codeine

Codeine, a central analgesic and cough suppressant, is metabolized in the liver and excreted principally in the urine. The two major metabolic pathways of codeine are glucuronidation and *N*-demethylation, whereas its minor pathway is the CYP2D6-mediated *O*-demethylation by which codeine is demethylated to generate its pharmacologically active metabolite, morphine (accounting for approximately 10% of administrated codeine). As expected, the analgestic effects of codeine were shown to be markedly reduced in patients who were CYP2D6 PMs [44,45]. Conversely, higher amounts of morphine were generated from either codeine [46] or two other of its analogs – oxycodone and hydrocodone – that require CYP2D6-catalyzed bioactivation [47] in CYP2D6 UMs, leading to an increased response to normal doses, or, in some cases, severe toxicity [46–48].

Targeted cancer therapy

Tyrosine kinase inhibitors

Relative to traditional cytotoxic chemotherapy, currently emerging targeted cancer therapy is based on mechanisms that target specific molecular abnormalities of tumor cells [49–51]. Thus, molecular-target dependence and patient selection are essential to the development of such targeted therapy [52,53]. For example, when activated by acquired somatic mutations or overexpressed, increased tyrosine kinase (TK) activity of the transmembrane epidermal growth factor receptor (EGFR, also known as ErbB1 or human epidermal growth factor receptor 1) plays a critical role in the proliferation and metastasis of tumor cells. Currently available EGFR-targeted antitumor drugs were designed to aim at such targets, resulting in the selective inhibition of critical molecules and specific signaling pathways that are involved in tumor growth and progression and are not required for normal cells (see below). Gefitinib (Iressa®), a TK inhibitor, is one of the first agents designed to specifically inhibit the activation of TK activity of the EGFR through the competitive binding of the adenosine triphosphate (ATP)-binding domain of the receptor, resulting in a remarkably rapid and often dramatic clinical response in a subgroup of patients with non-small cell lung cancer (NSCLC) [49,50,52]. Retrospective analyses suggested that gefitinib is more likely to be effective in Asian patients, females, nonsmokers and individuals with adenocarcinomas of the bronchioloalveclar carcinoma (BAC) subtype [54–59]. When compared with the patients who were nonresponders to gefitinib [55,57,59,60] or erlotinib (Tarceva®) [60], identical somatic mutations, such as short, in-frame deletions (relatively common) and single amino acid substitutions (such as *L858R*), have been identified more frequently clustered near the ATP-binding pocket of the TK domain of the EGFR in tumor specimens versus matched normal tissues from different gefitinib-sensitive patients. To assess the functional significance of the identified mutations, the wild-type and mutant receptors were expressed and their functions compared in cultured cell lines. The mutant EGFR receptors, *L858R*, and del *L747_P753insS* (del *L747_S752*, *P753S*), were shown to exhibit a greater amount and duration of the TK activation induced by EGF and also increase sensitivity to inhibition by gefitinib [55], with ten- to 100-fold higher sensitivity than the wild-type receptors [57,59–62]. The presumed molecular mechanisms underlying such hypersensitivity may be that mutant EGFRs have a more stable interaction between the TK and the drug than the wild-type receptors, resulting in increased inhibition of TK activity by the drug [57]. Many lines of evidence have demonstrated that the major EGFR signaling pathway – phosphatidylinositol-3 kinases (PI3Ks)/Akt (a serine-threonine kinase) – is involved in response to gefitinib, since EGFR-activated Akt signaling activity was observed in approximately half of NSCLC patients, in particular those whose phosphorylation of Akt (P-Akt) is positive [63] and/or whose ErbB3 expression is abundant [64]. This is due to the fact that the mutant EGFRs also use BrbB3 (a defined potent link between EGFR activation and PI3K activity) to activate the PI3k/Akt signaling pathway, leading to increased mutant EGFR-transduced antiapoptotic (or cell survival) signals [61,65]. These results suggest that substantial tumor responsiveness to gefitinib in NSCLC cells carrying *EGFR* somatic mutations results from increased apoptosis [66]. Moreover, gefitinib can effectively inhibit phosphorylation of EGFR and its downstream targets, such as Akt and extracellular signal-regulated kinases (ERKs) in EGFR mutant cell lines [57,66]. This may explain why patients who carried EGFR mutations experienced prolonged disease stabilization, and thus had a lengthened period of survival after the use of gefitinib when compared with patients without EGFR mutations.

Erlotinib, another ATP-competitive inhibitor of the EGFR-TK, exerts similar antitumor effects in NSCLC patients as gefitinib, because erlotinib-sensitive lung cancers also contained similar or identical somatic mutations found in gefitinib-responsive tumors [60]. This strongly suggests that these mutated EGFRs expressed in lung tumor tissues are the specific targets for these TK inhibitors [67,68]. However, in addition to the shared *EGFR* mutations with gefitinib, high sensitivity to erlotinib may be associated with high copy number and/or expression levels of the *EGFR* gene, or low levels of phosphorylated PKB/Akt and CDK2 kinase activity, or both [69].

In addition to NSCLC patients bearing the known functionally-important mutations, a subset of patients with ovarian cancer were recently reported to be more likely to respond to gefitinib. However, all observations of potential determination of tumor responsiveness to gefitinib or erlotinib in the above clinical settings require a large-scale prospective validation.

Trastuzumab & cetuximab

The human epidermal growth factor receptor (*HER)2* (ErbB2) gene is amplified in up to 30% of patients with breast cancer, resulting in the overexpression of the HER2 receptor protein that serves as the target for the anti-HER2 antibody trastuzumab (Herceptin®), a humanized monoclonal antibody [70]. The response rate to the antibody is estimated to be up to 35% in breast cancer patients with strong overexpression of HER2, while the drug is ineffective in the two-thirds of patients who do not overexpress the drug's target [70].

Similar to trastuzumab, the chimeric neutralizing antibody cetuximab (Erbitux®) – a monoclonal antibody that binds to the extracellular domain of EGFR – is able to specifically block the EGFR-mediated signaling pathway, thus preventing ligand activation of the receptor. Therefore, its clinical benefit manifests in certain tumors that overexpress EGFR, such as metastatic colorectal carcinomas [71], in particular those that lack somatic mutations in *EGFR* [72–74]. Cetuximab synergistically potentiates the activity of TK inhibitors and reduces the 50% inhibitory concentration (IC_{50}) values of gefitinib up to tenfold in the combinational targeted therapy [72]. Recent studies have indicated that cetuximab has a similar inhibitory effect on the growth of NSCLC cells with wild-type EGFRs compared with gefitinib, but has lower levels of apoptosis, and thus is less effective in suppression of the growth of EGFR mutant cells than gefitinib [66]. This suggests that cetuximab has relatively little inhibition of EGFR-mutant NSCLC cells, including those that later develop a secondary somatic mutation T790M in *EGFR*, because it is unable to inhibit phosphorylation of the mutant EGFRs [66].

Imatinib

Chronic myeloid leukemia (CML), a pluripotent hematopoietic stem cell disorder, is characterized by the Philadelphia (Ph) chromosome translocation t(9;22), resulting in the production of the breakpoint cluster region-abelson (*BCR-ABL*) fusion gene (an oncogene) that encodes a cytoplasmic chimeric protein with constitutive TK activity. Imatinib (Gleevec®) is a competitive inhibitor of ATP binding to the ABL kinase, a nonreceptor TK, thus inhibiting the constitutively activated BCR-ABL tyrosine kinase and producing rapid and durable clinical responses in CML patients with minimal toxicity [75]. In addition, imatinib is also an inhibitor of the C-KIT tyrosine kinase [50]. Based on this, the US FDA approved that imatinib can be used to treat BCR-ABL-positive CML and gastrointestinal stromal tumors (GIST) associated with activating mutations in *C-KIT*.

Other potential targets & drugs

Several new drugs targeting specific molecules and pathways are being developed. For example, an antibody that inhibits BrbB3's heterodimerization with other ErbB family members could be used to treat cancers driven by either EGFR or ErbB3, because ErbB3 is shown to mediate PI3K activity in gefitinib-sensitive NSCLC cell lines [64]. In addition, nonrandom somatic mutations in the *PIK3CA* gene that encodes the catalytic subunit p110α of PI3K, such as E542K, E545K and H1047R, are shown to possess increased catalytic activity, thus inducing rapid oncogenic transformation *in vitro* and *in vivo* [76]. As expected, a potent PI3K inhibitor (LY-294002) can effectively inhibit the growth of NSCLC cell lines [64].

Drug resistance

Clinical resistance to small molecule inhibitors targeting oncogenic TK is a common cause of cancer therapy failure. Three different mechanisms may be responsible for such resistance [53]:

- Target-dependent resistance (due to overexpression or secondary somatic mutations of the target genes, for example, the T790M mutation of *EGFR* [68,77–80])
- Target-independent resistance (due to independence of the TK activity, resulting in primary treatment failure, for example, *K-ras* mutations [81] and epithelial membrane protein-1 [EMP-1] expression [82])
- Drug-dependent resistance (due to alterations of drug transporters or drug-metabolizing enzymes [83])

Of these mechanisms, a marked advance in the study of target-dependent resistance has been made. Mutant kinases resistant to imatinib, gefitinib and erlotinib, such as T315I in BCR-ABL, T670I in C-KIT, and T790M in EGFR, are shown to weaken or prevent drug binding to a highly conserved 'gatekeeper' threonine residue near the kinase active site [84]. Based on this, some existing clinical compounds have been found to effectively inhibit these drug-resistant kinase variants and could perhaps be used to treat patients who have acquired resistance to first-generation targeted therapies [78,84]. For example, clinical resistance is increasing with prolonged use of imatinib [85,86]. Drug resistance is the result of the mutations (25 amino acid substitutions currently known) within the *BCR-ABL* kinase domain known to interfere with drug binding and, to a lesser extent, either amplification of the *BCR-ABL* gene [87] or reduced BCR-ABL dependence [88], thus, a more potent ATP-competitive inhibitor than imatinib is required for drug resistance. Dasatinib (formerly BMS-354825, a dual Src/ABL kinase inhibitor) is such a second-generation compound with increased potency, able to treat most imatinib-resistant patients with CML, except those who carry the T315I mutation (15–20% of all the resistant cases) [89]. Moreover, saturation mutagenesis screening of dasatinib-resistant *BCR-ABL* subclones identified ten different missense mutations at six different residues, four of which (L248, V299, T315, and F317) are dasatinib contact residues [88]. Among them, T315I confers the greatest degree of resistance to dasatinib with an IC_{50} value more than 750-fold greater than wild-type *BCR-ABL* [88], whereas mutant T315A and F317V have the next highest IC_{50} values, 40- to 90-fold higher than those without mutations. In contrast, other mutations conferring resistance, such as L248R, E255K, F317L, and V299L, display reduced sensitivity to dasatinib [88]. However, combination therapy with imatinib and dasatinib may delay the emergence of resistance [88].

A large number of structure–function studies suggest that most secondary somatic mutations in the kinase domain of the target kinases confer acquired resistance to the drug. In fact, in the case of NSCLC, the T790M mutation in EGFR (that exhibits reduced inhibition by the drug [77–79]) accounts for only a small fraction of cells within the recurrent tumor from some, but not all, patients with drug resistance [78,90]. This suggests that other mechanisms also underlie acquired resistance, or coexist with the T790M-associated resistance. Recently, EMP-1 – a cell surface-adhesion molecule – has been identified as a potential biomarker for drug resistance, both *de novo* and acquired. Its expression correlated well with nonresponse to gefitinib and is not associated with the somatic mutations of the EGFR kinase domain [82]. Remarkably, testing for the *EMP-1*-positive tumor tissues may help predict which patients would not benefit from therapy with gefitinib. In addition, increased internalization of ligand-activated EGFR (as measured with altered receptor trafficking) can also cause acquired drug resistance in patients without the T790M mutation [78]. Interestingly, in gefitinib-resistant NSCLC cell lines, the expression of ErbB3 was shown to be undetectable, indicating that these cells do not use ErbB3 to activate the PI3K/Akt pathway [64], whereas knockdown of ErbB2 causes a loss of cell viability, suggesting a role played by EGFR–ErbB2 heterodimers in transducing essential survival signals [78]. The latter may be used to explain why some dual EGFR/ErbB2 kinase inhibitors, such as HKI-272, EKB-569 and HKI-357, possess persistent suppression of the growth of gefitinib-resistant NSCLC cells bearing the T790M mutation [78]. Obviously, mechanisms underlying drug resistance are heterogenous (varying among individuals). For patients with cancer treated with molecularly targeted therapies at the time of relapse, rebiopsy and repeated genotyping of the tumor tissue are needed to improve drug efficacy.

Genetic susceptibility to diseases

Diseases are currently diagnosed mainly by their clinical phenotypes, with little reference to their mechanisms, although most common diseases have, to a varying extent, genetic susceptibility. Identification of distinct disease subtypes (each having identical or similar phenotypes) by genetic analyses can subclassify diseases and,

Figure 1. The Roche AmpliChip™ CYP450 Array.

The Roche AmpliChip™ CYP450 Array was designed to analyze two genes, *CYP2C19* and *CYP2D6*, that encode drug-metabolizing enzymes involved in the metabolism of approximately 25% of all prescription drugs. Reproduced with permission from Roche Diagnostics (Basel, Switzerland).
CYP: Cytochrome P450.

thus, patients. Increasing evidence shows that each patient's disease is molecularly unique, although the final phenotype may appear similar among different patients with an identical clinical diagnosis (for example, hypertension). Molecular or genetic subclassification of a certain disease has successfully guided the selection of drugs and their doses, as demonstrated by the following important examples. For instance, for patients with breast cancer, only those who have the amplified *HER2* gene are anticipated to respond well to trastuzumab [91]. Interestingly, for the same drug, its clinical outcomes (efficacy or resistance) vary by different mechanisms or molecular targets. For example, detection of the Ph^+ chromosome in patients with CML is a reliable predictor of the responses to imatinib [75,85]. However, the *T315I* mutation in the *BCR-ABL* gene was shown to determine clinical resistance to this drug [87], whereas some gain-of-function mutations in the *C-KIT* gene can be used to predict the responsiveness of GIST to imatinib [92,93]. Accordingly, subclassifying diseases with similar or identical phenotypes by molecular genetic techniques is essential to not only individualized drug therapy, but also individualized drug research and development [94], since important clues that will be used to develop drugs specific for a certain subgroup of patients can be found relatively easily from a subgroup of highly homogenous individuals with similar or identical disease susceptibility.

Future perspectives

Our current understanding of genetic variability does not yet allow us to fully interpret the outcomes of specific drug treatment, in particular for common diseases. The reasons for this are:

- The pathogenesis of most diseases is polygenic in nature
- Most drugs exert their actions or effects in a multigenetic manner
- There are complex interactions between the genes, disease, environment, drugs, and even pathogens (such as viruses, bacteria, parasites, and tumor cells)

In addition, the individual's genetic makeup is not the only determinant of variable drug responses, which are also influenced by factors such as age, gender, smoking habit, and other factors.

The field of pharmacogenomics is growing rapidly, bringing true personalized medicine closer to reality. New technologic developments have led to a vast increase in available data. This can be illustrated by a number of recent milestones:

- The number of pharmacogenomics topic-relevant publications is growing exponentially [19,95], illustrating an explosion of new information and knowledge.
- In January 2005, the FDA approved Roche's AmpliChip™ CYP450 Test for the rapid genotyping of *CYP2D6* and *CYP2C19* variants for diagnostic use [96,202]. Beginning in June 2005, physicians in hospitals and clinics were able to order and use the AmpliChip CYP450 Array (**Figure 1**) to predict how a patient will respond to a particular medication, and then personalize medicine for that patient.
- In February 2005, the first phase of the International HapMap Project [203] was completed. This project is anticipated to speed up the discovery of the genes and their variations involved in common diseases and individual variability in response to drugs,

Highlights

- Personalized medicine focuses on individualized drug treatment according to each patient's molecular diagnosis and genetic makeup.
- Individualized drug therapy involves optimal drug selection and rational dosage adjustment.
- Although not all drugs can be personalized, there is most likely to be clinical significance in tailored medicine for prodrugs, drugs with a narrow therapeutic index and drugs that target a key molecule or a critical pathway.
- Drug safety is the first arena in which patients can benefit from pharmacogenetics and pharmacogenomics.
- Tumor responses to the inhibitors of oncogenic tyrosine kinases are associated with the presence of activating mutations within the genes encoding the target kinases, targeted cancer therapy is thus a promising individualized drug therapy.

and help find new targets for therapies, leading to a better understanding of the molecular signals of disease and biologic basis for variable drug response.

- In March 2005, the FDA released its final version of the 'Guidance for Industry: Pharmacogenomic Data Submissions' [204], clarifying what type of pharmacogenomic data needs to be submitted to the FDA, and when. In addition, the guidance, for the first time, encourages the voluntary submission of data to the FDA. These so-called voluntary genomic data submissions (VGDS) are providing a novel platform for a scientific exchange of data that is outside of the normal regulatory review process, and can be used to share exploratory and research data that is not used for regulatory decision making.
- Last year, the US National Human Genome Research Institute (NHGRI) launched a 10-year program aimed at reducing the cost of sequencing mammalian genomes to US$100,000 in 5 years and finally to US$1000 per genome 5 years later [97] – the so-called '$1000 genome' [98,99]. As expected, a new DNA sequencing technology has been established recently [100], by which the cost of sequencing was roughly one-ninth that of the conventional technology (whose cost is currently estimated to be US$20 million for a human genome).

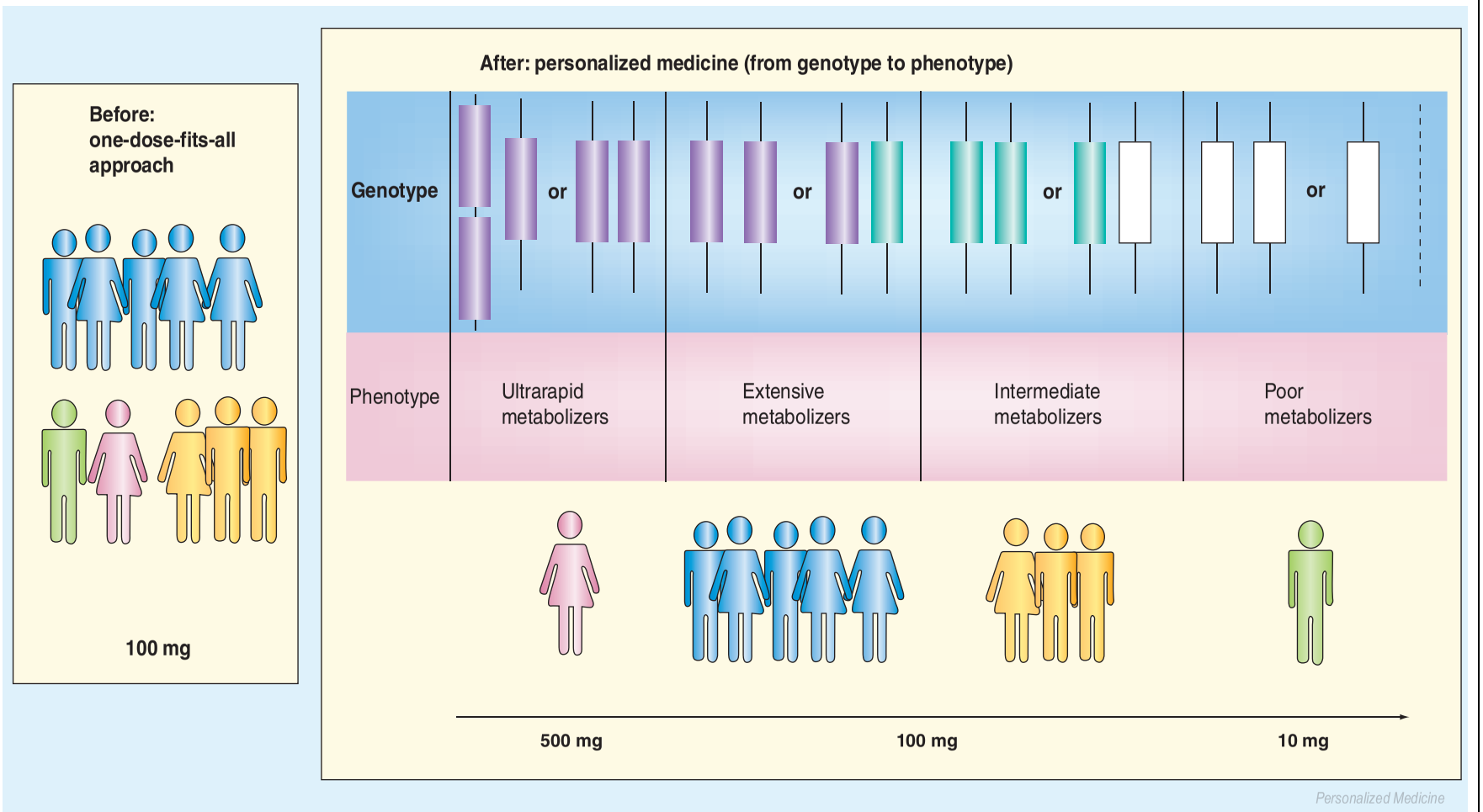
Clearly, a new era of personalized medicine is already under way. Although a large number of significant advances in pharmacogenetics/genomics studies are emerging, there is a very slow translation of pharmacogenomic information into patient care [18,19,27,101], and pharmacogenetic testing for drug metabolizing enzymes is still rarely performed in clinical practice, as demonstrated by a recent survey [27]. The following reasons may explain such a slow acceptance of pharmacogenomics in clinical settings [18,19,22,101,102]:

- There is a relative resistance to using genetic testing to individualize drug therapies, because conventional trial-and-error and one-size-fits-all approaches to prescribing medicines have long been widely accepted by most practicing physicians who graduated before the era of the human genome
- There is a lack of large-scale prospective clinical evaluations for the impact of genetic variability in drug disposition and response. In general, determining whether, or how, to use a pharmacogenetic test to predict the ability of an individual patient to metabolize, transport, and respond to a given drug will require reliable prospective genotype-to-phenotype evaluation
- There are additional costs for genetic testing

Accordingly, medical students and practicing physicians must be educated and trained to use pharmacogenetic tests and properly interpret their clinical relevance [95,103–107]. To address the need for education, the FDA has created a new training program for genomics with a self-teaching tutorial [204]. The Personalized Medicine Coalition has been founded [108,205], by which important information on pharmacogenomics and personalized medicine is updated daily. The American Academy of Family Physicians [206] has launched a series of workshops with the US National Institutes of Health [207] and the Centers of Disease Control and Prevention [208], educating the nation's 90,000 family doctors on how to use genetic information for patient care. Primary care physicians and specialists have also recognized a growing need to improve patient care by improving their understanding of pharmacogenetics/genomics. The US National Coalition for Health Professional Education in Genetics [209] has been formed to provide resources of continuing medical education for physicians and others in healthcare.

New, targeted drug therapies will become available and, with the rapid accumulation of new knowledge of pharmacogenomics [201,203–205,207,210,211], we anticipate that patient care will move from the hit-and-miss or one-size-fits-all approach to the treatment of each patient on an individual basis (**Figure 2**). Pharmacogenomics is playing an increasingly

Figure 2. Representation of the trial-and-error or one-dose-fits-all approach versus personalized medicine.



Differences in prescribing the same drug (nortriptyline, an antidepressant drug) before and after genotype-based medicine. The left panel shows a one-dose-fits-all approach. In the right panel, fully functional alleles of the *CYP2D6* gene are illustrated by purple boxes, functional but impaired alleles (such as *CYP2D6*2*, **9*, **10*, **17*) by green boxes, nonfunctional alleles (such as *CYP2D6*3*, **4*, **6*) by white boxes, and deletion of the CYP2D6 whole gene (i.e., *CYP2D6*5*) by a dashed line. The phenotype is predicted for each individual patient. To achieve the same plasma drug level, nortriptyline dose requirements (mg/day) vary by genotype [19].

important role in personalized medicine and we need to ensure that appropriate measures are being taken by all stakeholders to bring this exciting and clinically relevant knowledge to the patient.

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Note added in proof

Almost at the same time as when a cited new DNA sequencing technique was published [100]*, the other new alternative method was also released to reduce time and cost for sequencing* [109]*, reading 25 million bases of genetic code at 99% or better accuracy within four hours [109,110]. More recently, Japanese companies announced that they have built a desk-top machine by which any health worker could test a drop of blood for a particular genotype and get such a genotyping result in 1 hour for use in tailoring treatment to an individual's genes* [111].

Disclosure

The views expressed are those of the authors and may or may not represent the views of the US Food & Drug Administration (FDA). The contents of this document are not binding to the regulated industry or the FDA.

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