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Review

Pharmacogenetic testing: proofs of principle and pharmacoeconomic implications

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Abstract

Several proofs of principle have established that pharmacogenetic testing for mutations altering expression and functions of genes associated with drug disposition and response can decrease the "trial-and-error" dosing and reduce the risk of adverse drug reactions. These proofs of principle include thiopurine methyltransferase and thiopurine therapy, dihydropyrimidine dehydrogenase/thymidylate synthase and 5-fluorouracil therapy, folate enzyme MTHFR and methotrexate therapy, UGT1A1 and irinotecan therapy and CYP450 2C9 and S-warfarin therapy. These evidences advocate for the prospective identification of mutations associated with drug response, serious adverse reactions and treatment failure. More recent evidence with the HLA basis of hypersensitivity to the retroviral agent abacavir demonstrates the potential of pharmacogenetic testing and its pharmacoeconomic implications. With the convergence of rising drug costs and evidence supporting the clinical benefits of pharmacogenetic testing, it will be important to demonstrate the improved net health outcomes attributed to the additional costs for this testing.
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Keywords: Pharmacogenetics; Pharmacoeconomics

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1. Introduction

Over this last decade, with the rapid development of cost-effective high throughput molecular genotyping methods, pharmacogenetics has become increasingly important because of its potential to identify patients with increased risk of adverse side effects or decreased likelihood of response at standard dosage of drug. Many investigators have suggested that the addition of pharmacogenetic testing to the routine clinical practice can be extremely helpful because of the cost reduction associated with the identification of patients that will not respond to expensive drugs or to the identification of patients likely to suffer from severe adverse events. There are also tremendous efforts in the pharmaceutical industry to lower the cost for drug development and pharmacogenetics may fulfill the need to provide the right drug to the right patient and to increase the likelihood of success of large phase II and III clinical trials.

Pharmacogenetics, a term suggested by Vogel in 1959 [1] is the science that studies the relationship between genetic polymorphisms and drug response. Evidence of an inherited basis for drug response phenotypes dates to the 1950s, when Alving et al. observed that hemolysis during anti-malarial drugs treatment (primaquine, sulfonamides) was due to an enzymatic defect for the glucose-6-phosphate dehydrogenase [2]. Other observations included prolonged muscle relaxation after suxamethonium (succinylcholine) administration in individuals with an inactive form of cholinesterase [3], and an increased risk for neurotoxicity in individuals with a slow-acetylator phenotype receiving isoniazid therapy [4]. The first molecular defect was identified by Frank Gonzalez in the late 1980s. His group discovered that the molecular basis for the poor debrisoquine metabolizer phenotype in the livers of approximately 8% of most white populations was associated with deficiency in cytochrome P450 enzyme (CYP2D6) [5]. As CYP2D6 metabolizes many common drugs, this means that a significant proportion of the population is potentially at risk of side effects secondary to this defect.

The molecular basis of many pharmacogenetic polymorphisms has now been elucidated, and mutations resulting in alteration of expression or function of drug metabolizing enzymes or drug targets can occur in either the coding and non-coding regions (promoter, introns, 3′-untranslated region) [6,7]. It is now clear that every gene can present single nucleotide polymorphisms (SNPs) occurring in every 1000–3000 base pair throughout the human genome [8], and the current challenge is to elucidate which of these polymorphisms has relevance to drug treatment. In this communication, we will review the most relevant genetic polymorphisms associated with response to drugs.

2. Thiopurine methyltransferase and thiopurine therapy

Thiopurines (mercaptopurine, thioguanine and azathioprine) were developed in the 1950s by Gertrude Elion and George Hitchings, and are widely used as immunosuppressants in the treatment of inflammatory bowel disease [9,10], and as anti-proliferative agents in the treatment of acute lymphoblastic leukaemia [11,12] (Fig. 1).

Mercaptopurine, the analogue of hypoxanthine is activated intracellularly to thioinosine monophosphate (6-TIMP) by hypoxanthine phosphoribosyltransferase which is further converted to thioguanine nucleotides (TGNs) by a multistep process implicating inosine monophosphate dehydrogenase and guanosine monophosphate synthase [13]. This anabolic route is in competition with methylation of 6-TIMP into methylmercaptopurine nucleotides (6-MMPN) by polymorphic thiopurine methyltransferase. The intracellular activation of mercaptopurine is also in competition with two extracellular catabolic routes (oxidation of mercaptopurine to thiouric acid by xanthine oxidase, and methylation to methylmercaptopurine by TPMT). The anti-proliferative effects of thiopurines are associated with incorporation of deoxythioguanine nucleotides into the genomic DNA and de novo

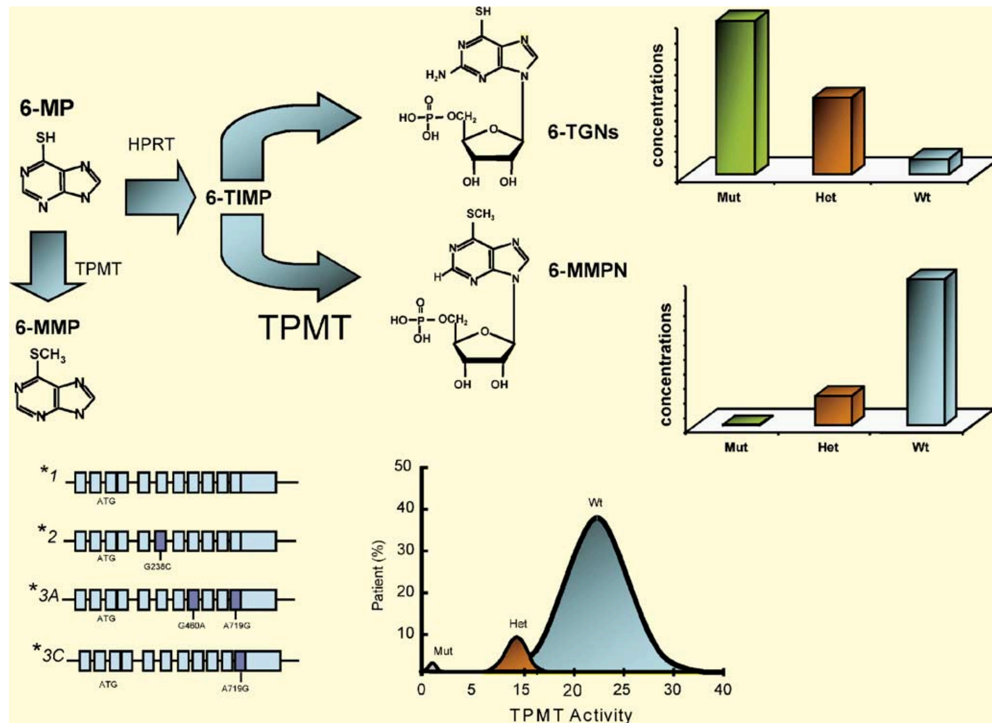

Fig. 1. Thiopurine methyltransferase and thiopurine therapy; mercaptopurine (6-MP) is activated to 6-thioinosine monophosphate (6-TIMP) by hypoxanthine phosphoribosyltransferase (HPRT), and subsequently converted to thioguanine nucleotides (6-TGNs) or alternatively methylated to methylmercaptopurine nucleotides (6-MMPN) by thiopurine methyltransferase. Three common mutations in TPMT gene (G238C, G460A and A719G) result in a trimodal distribution of TPMT activity. Individuals homozygous mutants for TPMT have higher intracellular 6-TGNs and lower 6-MMPNs concentration compared to those with a wild type genotype. This result in an increased risk of myelosuppression due to overproduction of TGNs in patients homozygous mutants. Conversely, increased 6-MMPNs production in patients with the wild type activity results in increased risk for hepatotoxicity.

purine synthesis inhibition by TGNs and 6-MMPN [14]. In contrast, the immunosuppressive effects of azathioprine appear to be associated with modulation of RAC-1 activity by thioguanosine triphosphate [15].

Pioneer work by Weinshilboum and Sladek in the late 1970s demonstrated that TPMT activity was polymorphic and transmitted as an autosomal co-dominant trait [16]. About 1 in 300 individuals cannot produce functional TPMT enzyme, 10% of the population is heterozygous for this polymorphism, and have intermediate levels of TPMT activity. The remaining 90% carry two wild type alleles and have full TPMT activity. TPMT has no known endogenous substrates, and individuals with defective TPMT activity are not known to exhibit any phenotype in the absence of drug treatment with thiopurines. The molecular basis for TPMT deficiency was discovered by Evans and co-workers in the 1990s at St Jude Children's Research Hospital (Memphis, TN) [17,18]. Three non-synonymous SNPs account for over 90% of the clinically relevant TPMT mutations [17–19], and the resulting amino acid substitutions do not affect the levels of TPMT transcript, but render the protein more susceptible to destruction through ubiquitination [20,21].

Mercaptopurine and azathioprine therapy can result into myelosuppression but patient carriers of the homozygous mutant TPMT genotype can experience life-threatening myelosuppression after normal doses of thiopurines, and can require dosage reductions of up to 10-fold in order to tolerate therapy [11,22]. More commonly, patient carriers of heterozygous mutations require dosage reductions of 10–50% [12,23] but can be also at risk of severe toxicity [24]. Most patients are treated with a "trial-and-error" approach to dosage

adjustments and this can lead to highly toxic adverse events including death in patients with TPMT deficiency [25]. TPMT genotyping would help prevent these severe adverse events and allow for individualization of therapy. In fact recent evidences have established the cost effectiveness of screening for thiopurine S-methyltransferase polymorphisms in patients with treated with azathioprine [26,27]. Testing for TPMT deficiency is currently offered by Prometheus Laboratories (San Diego, CA).

Patients with acute lymphoblastic leukemia with at least one mutant TPMT allele tend to have an improved response to thiopurine therapy and better chances of being cured, compared to patients with two wild type TPMT alleles [28]. This may be because they produce higher levels of TGNs. In contrast, patients having a normal TPMT genotype are less likely to experience severe side effects but may be at higher risk for therapeutic failure and adverse events secondary to the overproduction of 6-MMPN (hepatotoxicity) [9,29–31]. Thus investigators have advocated the measurement of TPMT activity to identify patients with high TPMT activity that may require more aggressive dosages or alternate therapy. However, TPMT on its own does not predict all toxicities to thiopurine therapy, and it is likely that additional SNPs in the purine pathway may affect function and expression of key enzymes implicated in the anabolism of thiopurine metabolites. For example, a common polymorphism in ITPase (inosine triphosphate pyrophosphohydrolase) which hypothetically converts thioinosine triphosphate to thioinosine monophosphate was shown to be associated with the occurrence of side effects to secondary to azathioprine in patients with inflammatory bowel disease [32]. Also, genetic polymorphisms in RAC-1 gene may alter the expression of the protein thereby modulating the effects of thioguanosine triphosphate [15].

3. Folate enzymes and methotrexate therapy

Methotrexate (MTX) is a folic acid antagonist widely used to treat cancer and immunosuppressive disorders such as rheumatoid arthritis. The mechanism of action of methotrexate is related to the inhibition of several enzymes implicated in folate metabolism (e.g., dihydrofolate reductase), critical for nucleotide and amino-acid synthesis [33]. MTX enters cells through the reduced folate carrier (RFC-1) and is activated by folylpolyglutamate synthase to methotrexate polyglutamates (MTXPGs) [34–37]. This γ-linked sequential addition of glutamic acid residues enhances the intracellular retention of MTX, and promotes the adenosine release through inhibition of amino-imidazole carboxamide (AICAR) transformylase, the last enzyme in the de novo purine synthesis pathway [38–40] (Fig. 2).

The enzyme 5,10-methylenetetrahydrofolate reductase gene (MTHFR) is involved in maintaining folates and homocysteine homeostasis, and deficiencies of MTHFR are implicated in neurological and vascular diseases [41]. A common genetic polymorphism in MTHFR [42] consisting of a C–T transition at nucleotide 677 is associated with decreased MTHFR activity and altered folate levels [41,43,44]. Individuals with a homozygous mutant TT, or heterozygous CT genotype (∼10% and 40% of the population, respectively) present increased risk of side effects following MTX therapy compared to those with the wild type genotype [45–48]. The precise mechanism for this increased toxicity in patients with the 677T variant is not clearly understood but it can be speculated that lower folate levels in those patients may predispose to increase susceptibility to the anti-folate effects of MTX. This is supported by the observation that folate supplementation tends to overcome the effects of decreased MTHFR activity in patients with rheumatoid arthritis [48]. Testing for the C677T mutation in patients undergoing MTX therapy could be important as the dose is often escalated to achieve therapeutic response. Other polymorphisms in the reduced folate carrier (RFC-1 G80A), thymidylate synthase and in AICAR transformylase have been also recently been implicated in MTX responsiveness [49]. It is also likely that polymorphisms in MTX's target such as DHFR (T–C at position 829) [50] or in enzymes involved in the catabolism of MTX (γ-glutamyl hydrolase) or its efflux (such as MRP4) may also contribute to MTX response [51,52].

4. Dihydropyrimidine dehydrogenase, thymidylate synthase and fluorouracil therapy

5-Fluorouracil (5-FU, an analogue of uracil) is widely prescribed for the treatment of breast and

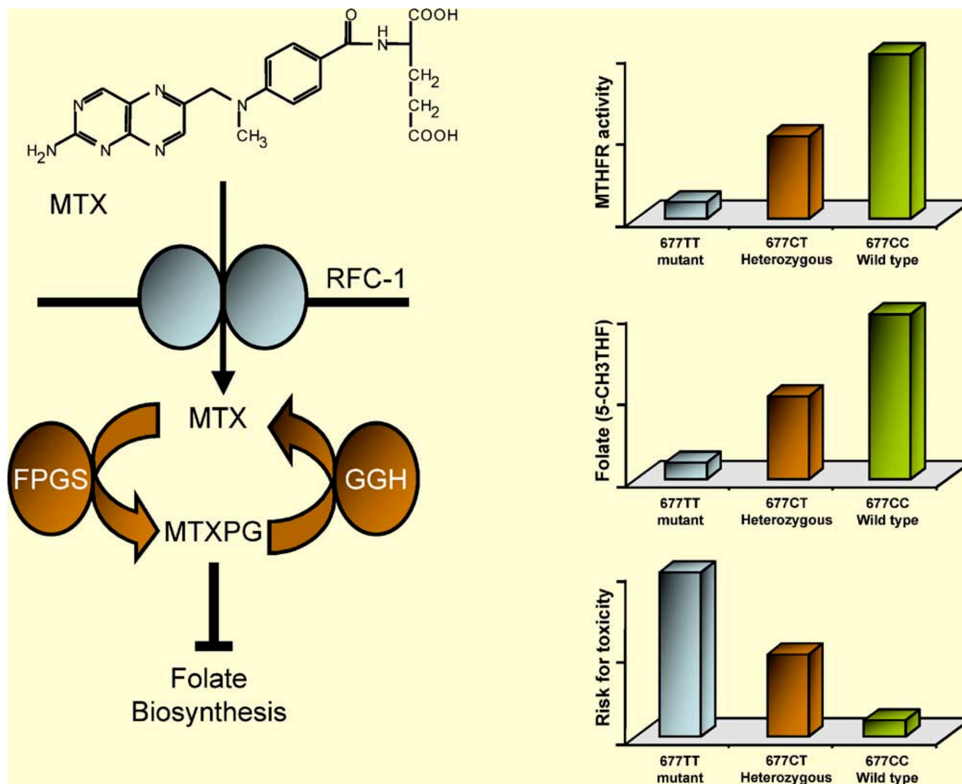

Fig. 2. MTHFR and methotrexate therapy; methotrexate (MTX) enters cells through the reduced folate carrier (RFC-1) and is activated to methotrexate polyglutamates by FPGS, a process in competition with deconjugation by γ-glutamyl hydrolase. MTXPGs inhibit folate biosynthesis. Patients with the MTHFR 677TT genotype have reduced MTHFR activity and reduced folate levels (5-methyltetrahydrofolate) compared to patients with MTHFR 677CC genotype. This result in a greater susceptibility to the anti-folate effects of MTX in patients with 677TT genotype compared to those with the 677CC genotype. This increased risk can be significantly reduced through folate supplementation.

colorectal cancer. 5-FU is a prodrug converted to 5-fluoro-2-deoxyuridine monophosphate (5-FdUMP), an inhibitor of thymidylate synthase (TS) in a ternary complex with 5-methylenetetrahydrofolate [53]. TS is an enzyme required for de novo pyrimidine synthesis, and its inhibition results into cell death [54]. In the liver, more than 80% of 5-FU is inactivated by dihydropyrimidine dehydrogenase (DPD) [55]. DPD activity varies from 8- to 21-fold among individuals [56,57] and patients with low DPD activity cannot efficiently metabolize 5-FU. The resultant is the formation of excessive amounts of active metabolites, which leads to severe hematopoietic, neurologic and gastrointestinal toxicities that can be fatal [58,59]. DPD is subjected to a common genetic polymorphism. Although the exact frequency of individuals with defective DPD alleles is not clear, the severe toxicity occurring after 5-FU therapy in patients with reduced DPD activity (below 100 pmol/min/mg protein in peripheral mononuclear cells) suggests that approximately 3% of individuals are heterozygous, and 1/1000 is predicted to be homozygous mutant for inactivating mutations [56,57,60]. Total DPD deficiency is an inborn error of pyrimidine metabolism and is often associated with thymidine-uraciluria leading to neurological disorders [61], whereas heterozygotes are less likely to exhibit a phenotype in the absence of drug challenge. A G–A transition at the exon 14 5′-splice consensus sequence characterizes about 50% of non-functional DPD alleles, and leads to skipping of exon 14 and synthesis of a truncated protein degraded by the ubiquitin–proteosome system

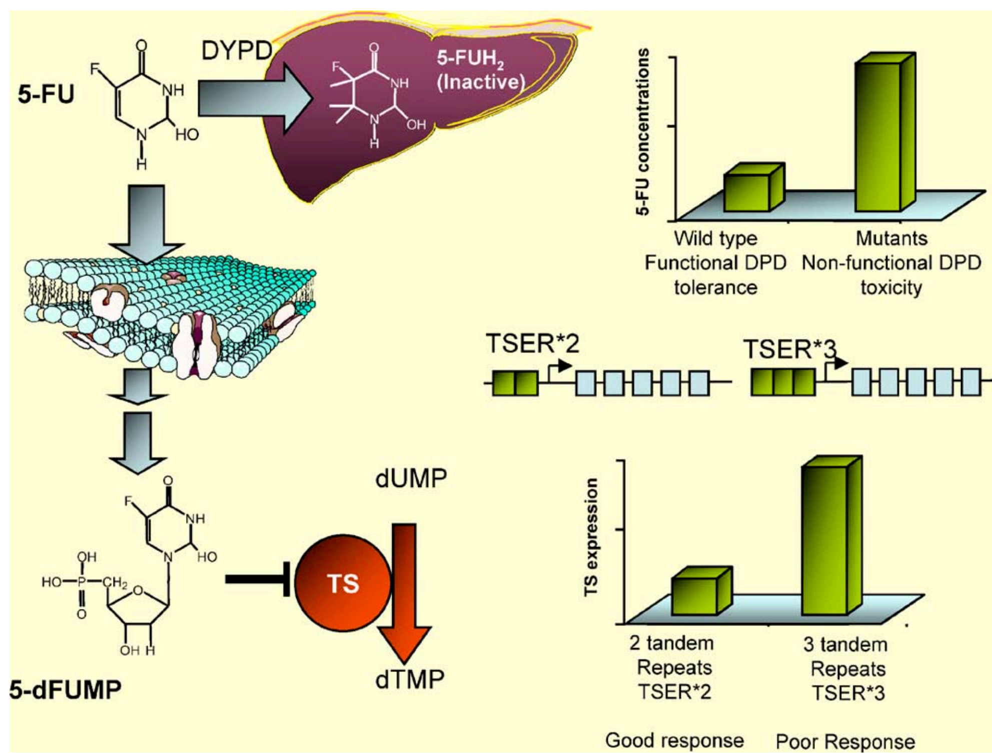

Fig. 3. DPD, TS and 5-FU therapy in the liver 5-FU is inactivated by dihydropyrimidine dehydrogenase (DYPD) into dihydrofluorouracil (5-FUH_2). Patients with non-functional DPD, cannot metabolize 5-FU and the shunt of 5-FU towards intracellular activation to 5-fluorouridine monophosphate (5-FUMP) results into increased risk of toxicity. 5-FUMP binds and inhibits thymidylate synthase (TS, which converts deoxyuridine monophosphate to deoxythymidine monophosphate) to produce effects. Patients with two tandem repeats in the TS enhance region of TS (TSER*2) have lower expression levels than those with 3 tandem repeats (TSER*3). This results into an improved response to 5-FU in patients with two tandem repeats compared to three tandem repeats.

[58,62,63] To date, a total of 17 mutations associated with reduced DPD activity have been reported [64]. Also, this example illustrates the challenge facing pharmacogenetic testing, as only cost effective and high throughput genotyping methods will allow the testing for these multiple mutations in the DPYD gene (Fig. 3).

A genetic polymorphism in TS [65] may also affect the outcome of 5-FU therapy [66,67]. In vivo and in vitro studies have demonstrated that low TS activity is associated with improved response to 5-FU compared to higher TS activity [68,69]. TS expression is regulated by a polymorphism characterized by a variable number of tandem repeats (2 or 3 repeats) in the enhancer promoter region of TS [70,71]. The presence of triple tandem repeats increases in vitro TS expression compared to double tandem repeats [70] and is associated with higher in vivo tumor TS activity [69]. Therefore, patients having a homozygous genotype with 3 tandem repeats (high TS activity) have a lower probability of response to 5-FU than patients with 2 tandem repeats and may be at lower risk of toxicity [66]. Furthermore, as 5,10-methylenetetrahydrofolate enhance the binding of 5-FU metabolites on TS, investigators recently demonstrated that the C677T mutation in MTHFR (mutants have increased intracellular levels of 5,10-methylenetetrahydrofolate) is associated with increased likelihood of response to 5-FU therapy [72]. Furthermore, because the 5,10-methylenetetrahydrofolate precursors enter the cells through the reduce folate carrier (RFC-1) it can be hypothesized that polymorphisms in RFC-1 are likely to affect folate homeostasis and thus response to 5-FU.

5. UDP-glucuronosyltransferase UGT1A1 and irinotecan therapy

Irinotecan is active in various solid tumors such as colon cancer and lung cancer [73]. Irinotecan is a prodrug requiring conversion by carboxylesterase to active SN-38, which inhibits topoisomerase I to exert anti-tumor activity [74]. Hepatic UDP-glucuronosyltransferase 1A1 (UGT1A1), whose endogenous substrate is bilirubin [75], inactivates SN-38 into the more polar SN-38 glucuronide, which is then eliminated in bile and urine [76]. The dose-limiting toxicities of irinotecan consist of diarrhea and leukopenia [76,77] and these toxic effects are associated with excessive formation of SN-38. Because UGT1A1 expression is highly variable, and in vitro studies have shown a 17- to 52-fold inter-subject variation in the rate of SN-38 glucuronidation [78–80], the inter-patient variability in response to irinotecan may be related to the rate of glucuronidation (Fig. 4).

The UGT1A1 gene is part of a large family of at least 12 UGT-glucuronosyltransferase enzymes encoded by the UGT1 locus on chromosome 2. The organization of the UGT1 locus is complex, and involves at least 12 alternative exons 1, each with their own promoter and common exons 2–5. Reduced expression of the UGT1A1 is often associated with increased blood levels of unconjugated bilirubin [81,82]. Variability in the number of TA repeats in the UGT1A1 promoter region [75] at the binding site for the transcription factor IID is one cause of reduced UGT1A1 expression that is manifested as Gilbert's syndrome, a mild chronic hyperbilirubinemia that often goes undiagnosed. The presence of 7 rather than 6 TA repeats in the UGT1A1 promoter reduces enzyme expression and also reduces levels of SN-38 glucuronidation [78], causing a higher

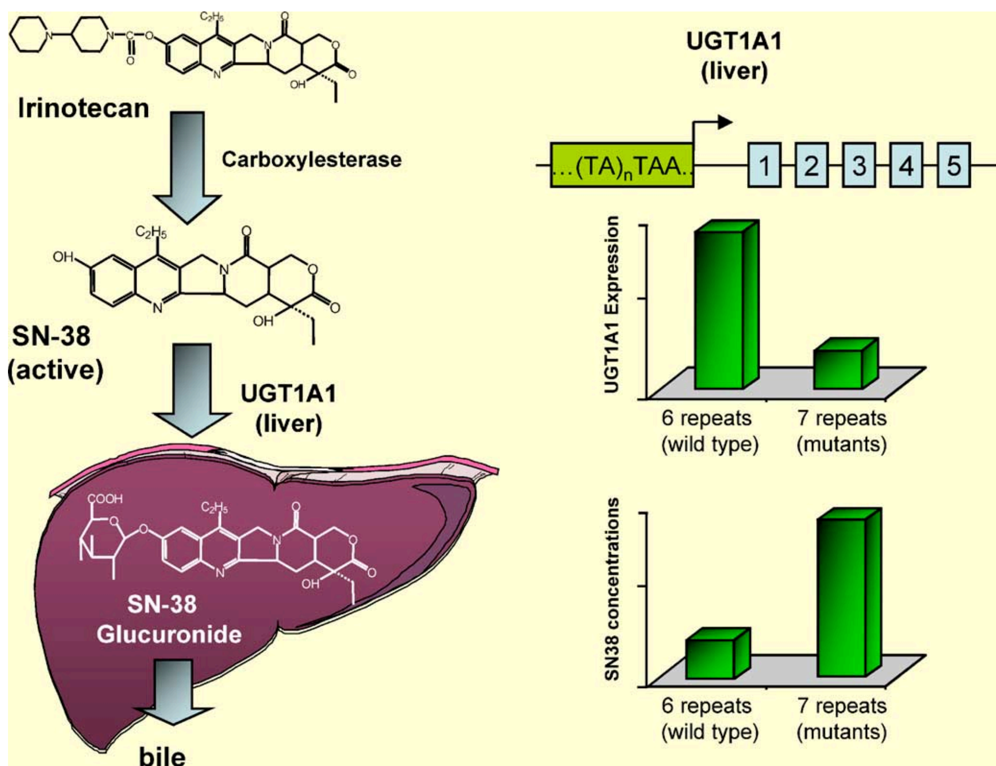

Fig. 4. UGT1A1 and irinotecan therapy; irinotecan is activated to SN38 by carboxylesterase to produce anticancer effects. In the liver SN-38 is inactivated to SN-38 glucuronide by UGT1A1. UGT1A1 expression is polymorphic and patients with six tandem repeats in UGT1 A1 have higher UGT1 A1 expression than those with seven tandem repeats. Subsequently, SN-38 concentrations are higher in patients with carriers of seven tandem repeats (vs. six tandem repeats). This results in a higher risk of developing toxicity at standard dose of irinotecan.

chance of developing diarrhea and/or leukopenia during irinotecan therapy [77,83]. Gilbert's syndrome is also associated with the TA7/TA7 genotype, [79] and these patients may be at increased risk of toxicity from irinotecan [77,84]. Polymorphisms in other genes such as carboxylesterase [85,86] and transporters (MRP1) [87] may also affect irinotecan pharmacokinetics and response.

6. Cytochrome P450 and warfarin therapy

Cytochrome P450 proteins (named for the absorption band at 450 nm due to the carbon monoxide binding spectrum), are one of the largest families of enzyme proteins and an important factor in phase I oxidative drug metabolism. Their amino acid composition is very diverse but their structure is highly conserved. The structure is centered around a heme moiety whose major feature associates a binding and catalytic function. The main catalytic function of CyP450 is the transfer of electrons from NADH to catalyze the oxidation of a large number of different substrates [88] (Fig. 5).

Pharmacokinetics is characterized by four phases (absorption, distribution, metabolism and excretion). Oxidative metabolism by cytochrome P450 enzymes is the primary mechanism of drug metabolism.

Warfarin and CyP2C9 illustrate the pharmacogenetic implication of CyP450 in drug metabolism. Warfarin (acilonylbenxyl hydroxycoumarim) is an anticoagulant acting as an inhibitor of vitamin K-dependent coagulation factor. Warfarin is racemic mixture of *R* and *S* enantiomers. The *S* form of the

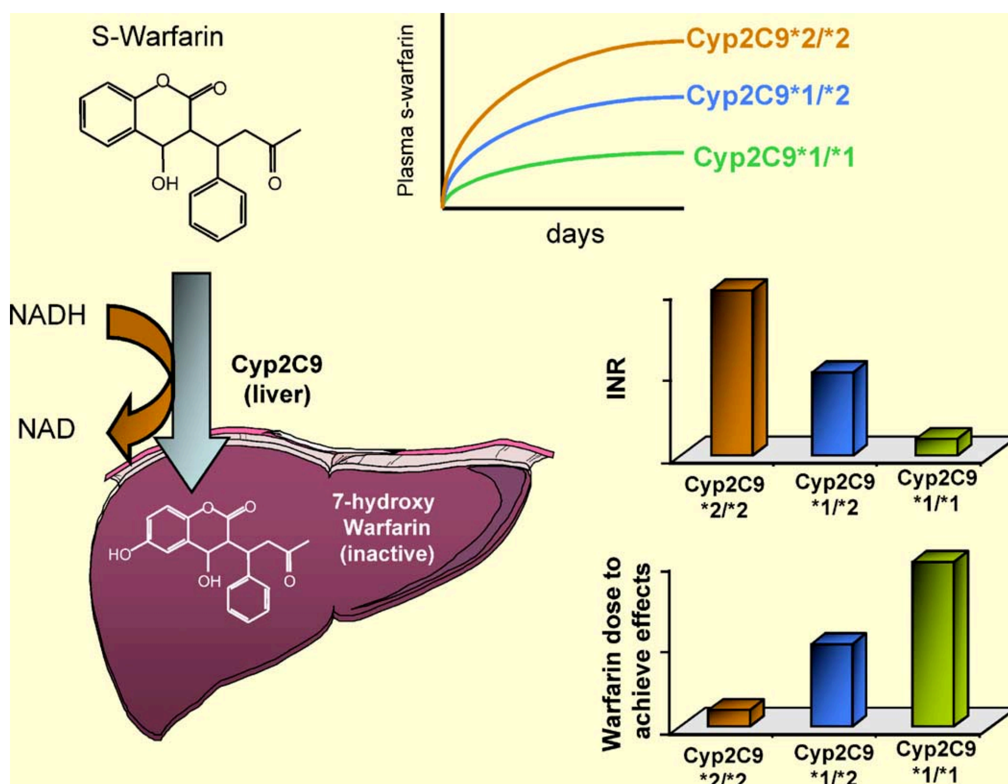

Fig. 5. CyP4502C9 and S-warfarin therapy; S-warfarin is oxidized to 7-hydoxy Warfarin by microsomal cytochrome P4502C9. Patients with a homozygous mutant genotype for CyP4502C9 *2/*2 have decreased hydroxylation capability compared to those with the wild-type CyP4502C9 *1/*1 and higher plasma concentration of active S-warfarin. This can results into greater anti coagulation effects (increased INR: international normalized ratio) before dose adjustment in those patients that require lower dose to achieve effects compared to those with the wild type genotype.

anti-coagulant is three to five times more potent than the *R* form [89]. The metabolism of the *S* form of the drug is therefore far more important than the metabolism of the *R* form of the drug when considering anti-coagulatory effects.

Cytochrome P4502C9 is the principal hepatic microsomal enzyme responsible for the metabolism of S-warfarin. The wild-type Cyp4502C9*1/*1 predominates in humans. However, several polymorphisms altering Cyp4502C9 expression are associated with risk of bleeding complications at standard dosage of warfarin. The presence of Cyp2C9 polymorphisms primarily Cyp2C9*2 and Cyp2C9*3 either heterozygous or homozygous is associated with a reduction in the metabolism of S-warfarin [89–94]. The increase in plasma levels results into increased anti-coagulation effects. In a study of 185 patients followed over a mean 2.3 years, the incidence of serious and life threatening bleeding was higher in those carrier of a Cyp2C9 mutant genotype compared to those with the wild type genotype [95]. Determining Cyp2C9 polymorphisms may help identify patients candidates for dosage reduction.

Several pharmaceutical companies have established projects to screen for polymorphisms in cytochrome P450 enzymes that will identify genetic profiles likely to predict adverse side effects prior to drug administration.

7. HLA typing and abacavir

Recently, two research teams have independently identified a genetic marker that predicts which HIV-infected patients are at risk of a hypersensitivity reaction to abacavir, a HIV-1 nucleoside reverse transcriptase inhibitor [96,97]. About 5% of patients treated with abacavir develop a severe, potentially life-threatening hypersensitivity reaction. Abacavir sensitivity usually starts with severe gastro-intestinal symptoms, followed by fever and rash. Discontinuation of the drug reverses symptoms but re-challenge with abacavir in hypersensitive patients can be fatal. In the Australian cohort study [96], patients with the HLA-B57 allele were 114 times more likely to experience a hypersensitivity reaction when treated with abacavir. Similarly, the Glaxo retrospective case-control study [97] revealed that 46% of patients with the HLA-B57 allele presented hypersensitivity to abacavir versus only 4% in the control group.

8. Pharmacogenetics and pharmacoeconomics implications

Innovations in pharmacogenetics rather than price regulation may provide an interesting solution to the rising costs of prescription as the role of genetics on drug metabolism and toxicity will dramatically change the practice of medicine over the next decade.

Since 1992, prescription drug prices in the United States have risen dramatically with the average price of drugs per prescription among the elderly rising 48%, consuming 14% of the average Society Security benefit and accounting for almost half of the total increase in healthcare costs [98,99]. However, increased usage and innovation, not price increases, appear to be the driving forces behind this increase in cost as IMS Health found that of the 17% increase in pharmaceutical costs in 2001 prescription sales, only 5% came from price increases [100]. In December 2003, the US Congress passed the Medicare prescription drug benefit that the Congressional Budget Office estimates will account for 50% of Medicare outlays over the next 10 years [101]. Prior to passage of this law, it was estimated that the Medicare Hospitalization Insurance trust fund would be running a cash deficit by 2013, when the first wave of 77 million “baby boomers” has retired. In light of this anticipated insolvency, a reduction in Medicare benefits and strict regulation of payments for prescription drugs is expected, similar to what has occurred to physician and hospital payments over the past 20 years.

Pharmacogenetics may reduce costly treatment failures and adverse events (that contribute to the increase in prescription drug costs) by prospectively predicting a patient’s drug activation and detoxification status in order to guide therapeutic intervention [102]. Additionally, some evidence suggests that genetic tests are better predictors of drug metabolism than ethnicity or geography [103]. Avoiding the pharmacy costs of treatment failures or severe adverse events would be a logical rationale to justify the additional medical costs of this type of testing. Unfortunately, pharmacy benefits and medical benefits are often analyzed independently; thus, it is difficult to quantify the trade off between more laboratory costs and reduced pharmacy costs.

Table 1
Definition of pharmacoeconomic terms

Term	Definition
Cost-minimization analysis (CMA)	Compares the effectiveness of two or more treatments in terms of clinical and quality-of-life (QOL) measures, with economic cost measured in monetary units being the only distinct factor (12)
Cost-effectiveness analysis (CEA)	Compares net monetary costs of a medical intervention with a measure of effectiveness (i.e. clinical or QOL) resulting from the intervention, and compares this ratio with those of other interventions (12)
Cost-Benefit Analysis (CBA)	Enumerates and compares the net costs of an intervention with the net benefits, or cost savings which arise from the intervention, to derive a ratio of total monetary cost divided by the benefits expressed as monetary savings in projected expenses (12)
Cost-utility analysis (CUA)	A type of CEA which incorporates the value of life in variables, by assigning values to various health outcomes to delineate the relative importance of the different kinds of health outcomes to people. These results are expressed in measurements such as cost per quality-adjusted life-year (QALY), which is the most commonly used unit (12)

How will pharmacogenetic testing provide improved clinical outcome at a reasonable additional cost compared to current practice? To review this question, we must review the four major types of analyses that could be employed to illustrate the economic benefits of this testing: cost-minimization analysis, cost-benefit analysis, cost-effectiveness analysis, and cost-utility analysis (Table 1) [104–106]. Each approach offers insights into the role of pharmacogenetics to provide a positive economic result.

In all of these analyses, the studies may involve comparing two treatment arms; one having its dosing and patient selection guided by pharmacogenetic testing and another more representative of an empiric standard of care. In light of the incremental costs associated with pharmacogenetic testing, it will likely be difficult to prove that such testing minimizes monetary units, but such studies may clearly show a net health outcomes benefit. For example, in certain circumstances where treatment failure results in shifting therapy from an inexpensive generic compound to an expensive branded agent, studies may illustrate a potential ability to minimize overall costs by extending the therapeutic segment for less-costly agents.

Managed care organizations remain skeptical of the positive health outcomes from pharmacogenetic testing. An example is the Blue Cross Blue Shield Technology Evaluation Center (BC/BS TEC) that has issued guidance on insurance coverage for chemotherapeutic resistance and susceptibility assays that include genetic markers related to common therapies such as fluorouracil (5-FU). The BC/BS TEC laid out a rationale that provides a road map for establishing the economic benefits of pharmacogenetic testing in the future.

First, the committee examined studies illustrating the ability of in vitro assay results to predict in vivo response to chemotherapy. They found that such an approach does not address the relevant comparison of whether clinical outcomes differ between treatments aided by an assay versus empiric treatments. The Committee went onto say, “Furthermore, a review of studies correlating in vitro assay results with in vivo response shows that a negative assay result can be false in 20% of cases and a positive assay result can be false in 13% of cases” [107].

Second, the committee reviewed decision analysis as a means of justifying clinical validity and reimbursement of these tests. Despite the wide use of decision analysis to systematically improve technology assessment when there is uncertainty about clinical or economic outcomes, there have been few published analyses examining the cost effectiveness of screening for enzyme polymorphism based strategies [108,109]. One such publication by Marra et al. involved modeling the cost effectiveness of 2 alternative azathioprine treatment strategies involving pre-prescription TPMT genotyping resulting in dose reductions and no testing with usual therapy [109] This study found that prospective pre-prescription TPMT genotyping to guide azathioprine dosing based upon the diagnosis of TPMT reduction and deficiency resulted in a small direct cost reduction compared to base case estimates. Since indirect costs were not included in this study, it is likely that these results understated the cost-savings achieved by the genotype-based dosing strategy. Another analysis from Hughes et al. found that pre-prescription genotyping for HLA B*5701 in HIV patients undergoing treatment including abacavir

is cost-effective to avoid hypersensitivity reaction, as well as the higher costs associated with alternative therapies in the HAART regimen [110]. Despite findings from decision analysis tools, the BC/BS TEC has dismissed similar efforts to perform decision analysis as an inadequate "tool for comparing assay-guided and empiric chemotherapy because the evidence is insufficient to estimate probabilities for all key outcomes and there is a high degree of uncertainty about the assumptions that should be made regarding how assays are conducted and used in treatment decisions" [107].

The only studies deemed viable by the BC/BS TEC were those attributing statistical significance to the outcomes of assay-guided treatment versus empiric treatment [107]. Thus, the BC/BS TEC suggests that randomized, prospective, controlled clinical trials must be designed to compare outcomes from at least two treatment arms, one that has treatment guided by the pharmacogenetic assay and one representative of empiric treatment. However, there are three critical challenges to this approach. First, with the low relative prevalence of many genetic mutations that contribute to serious adverse events, these studies may require a substantial number of patient enrollments in order to attribute statistical significance. Second, with the life-threatening nature of some of these toxicities, it may be very difficult to justify to an IRB that such a study is ethical when a patient having a mutation that is at high risk for toxicity may still be exposed to therapy. And third, it is sometimes very difficult to define "empiric treatment" or "standard of care" since patients with chronic diseases tend to be very heterogeneous and treatment patterns vary widely. To overcome these challenges, costly studies will need to be employed that involve three key factors: (1) large patient populations to account for the low prevalence of these genetic mutations; (2) protocols that mitigate the risk to patients in the event of serious adverse events, and; (3) systematic evaluations of treatment patterns using observational health claims data to justify a clinical study design defining "standard of care" or empiric treatment.

In summary, there is a need to conduct prospective, controlled clinical studies to establish evidence that treatment guided by genetic polymorphisms offers an improved net health outcome versus empiric treatment. By reducing costly treatment failures and serious adverse events, pharmacogenetic testing may help improve treatment for chronic diseases, reduce the burden of rising prescription drug costs, and lower the costs of drug development.

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