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Addressing The Challenges Of The Clinical Application Of Pharmacogenetic Testing

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Abstract

Pharmacogenomics aims to use molecular genetic markers to predict treatment outcome. Indeed within the past decade, there has been a rapid emergence of pharmacogenetic tests to aid clinicians to predict efficacy or toxicity for some drugs. Despite this major advance in therapeutic drug management there remain challenges to the appropriate use of pharmacogenetic tests. We discuss *UGT1A1* pharmacogenetic testing to illustrate the knowledge gaps impeding widespread use of pharmacogenetic tests in the clinical setting.

Introduction

Pharmacogenetic tests are potentially useful tools for making a therapeutic decision by identifying patients who should or should not receive a particular drug, or guiding individual drug-dosing. Pharmacogenetic test information is currently included in over 200 drug labels among those approved in the United States (1). The information is classified into three categories that guide the clinical use of pharmacogenetic tests for reaching a therapeutic decision (Table) (2): 1) test required, 2) test recommended and 3) information only. Thus far, only four drugs – cetuximab, trastuzumab, maraviroc and dasatinib – require a pharmacogenetic test before they are prescribed. Therefore the majority of drugs with labels containing pharmacogenetic test information do not require pharmacogenetic testing. For example, the label for the anticancer drug irinotecan recommends testing for the presence of a variant of UDP-glucuronosyl transferase 1A1 (*UGT1A1*) to prevent drug toxicity. However, pharmacogenetic testing for *UGT1A1* has many challenges for its appropriate clinical use, similar to most pharmacogenetic tests. In this paper, we use the *UGT1A1* pharmacogenetic test as an example to illustrate some of the major challenges to the clinical application of pharmacogenetic tests – incomplete knowledge of the extent of human genetic variation, availability of alternative biomarkers, and the lack of a model of delivery for pharmacogenetic information.

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UGT1A1 Pharmacogenetic Testing

UGT1A1 is a hepatic enzyme involved in the glucuronidation of bilirubin and many drugs such as the active metabolite of the anticancer drug, irinotecan. Enzymatic activity of UGT1A1 differs among individuals and can be indirectly affected by environmental, physiologic and epigenetic changes. However, UGT1A1 enzyme function is genetically determined by inherited sequence variation in the coding and non-coding regions, most notably a two base pair insertion $(TA)_n$ in its promoter region (3). The most common promoter variants are $(TA)_5$, $(TA)_6$, $(TA)_7$, and $(TA)_8$ (3). The $(TA)_5$ and $(TA)_6$ variants are associated with high UGT enzymatic activity; the $(TA)_6$ allele in particular is referred to as the *1 or "wildtype" allele. The $(TA)_7$ and $(TA)_8$ repeats are associated with low UGT enzymatic activity (3). For the purposes of this paper, we focus on the variant $(TA)_7$, or *28, allele because it has received the most attention.

The *28 allele is associated with inherited forms of unconjugated hypberbilirubinemia such as Gilbert syndrome and Crigler-Najjar syndromes (4). With regard to drug therapy, retrospective and prospective studies have demonstrated an association between the presence of reduced UGT1A1 enzymatic activity (especially with the *28 allele) and increased incidence of toxicity, most notably severe neutropenia in patients treated with irinotecan (5,6). The association between the *28 allele and severe diarrhea is not as statistically significant as is neutropenia. Therefore there may be other yet unknown genetic factors that are more predictive of irinotecan-induced diarrhea. In 2005 the FDA amended the package insert for irinotecan to include a recommendation, but not a requirement, to test for the *28 *UGT1A1* variant to predict those at risk for neutropenia (7). Shortly thereafter, the Invader® Molecular assay for *UGT1A1* genotyping appeared on the market as an FDA approved test, with an overall correct call rate of 98.8% (8).

A recent meta-analysis reports that the risk of severe neutropenia for patients homozygous for the *28 allele may be a function of the administered dose of irinotecan (9). The study found that risk of toxicity was higher for patients homozygous for *28 allele and on chemotherapeutic regimens, of irinotecan alone or in combination with other myelotoxic drugs, containing medium (125-150 mg/m^2 OR= 3.22, 95% CI= 1.52 to 6.81; P=0.008) and high dose irinotecan (> 150 mg/m^2 OR=27.8, 95% CI=4.0 to 195; P=0.005) compared to patients heterozygous for *28 and homozygous for *1 (wild type) (9). For this reason the study authors have asserted, contrary to the FDA's initial recommendation of a dose reduction based on genotype alone, that a reduced irinotecan dose or an alternative treatment regimen be used only when the *28 allele is present **and** a high dose irinotecan regimen is used (9). This recent finding may help refine the FDA labeling of irinotecan as pertains to the interpretation of the *UGT1A1* genotype within the context of appropriate dosing of irinotecan. However, recent reports show that a decrease in irinotecan dose for patients homozygous for the *28 allele may result in decreased tumor response, further complicating the appropriate drug-dosing to prevent irinotecan toxicity (8).

In light of the FDA's initial recommendation, oncologists at several academic health centers incorporated the *UGT1A1* test into their clinical practice. It is unknown, however, how knowledge of the genotype impacted on their care of the patient (8). On the other hand there are others still who did not heed the FDA's recommendation for *UGT1A1* genotyping, due in part to the uncertainty of its clinical utility. What then are the challanges to the use of pharmacogenetic testing for *UGT1A1* in the clinic?

The first challenge is that the *UGT1A1* test may be limited in its general applicability in **diverse** populations. There are at least 113 variants in the *UGT1A1* gene, most of which are associated with reduced or inactive enzyme activity, a couple associated with increased

activity, and still others are of unknown significance (4). However, the most widely used *UGT1A1* test (Invader® Molecular assay) only assesses one promoter variant, the *28 allele. Homozygosity for the *28 allele occurs in 10% of the North American population and at a similar frequency in Caucasians and African Americans, but less so in Asians (4). The frequency of the *28 allele has not been thoroughly studied in other ethnic groups, such as Hispanics living in North America. Even if one were to comprehensively test for all the known genotypes of the *UGT1A1* promoter polymorphisms, the mere fact that only that portion of the gene is assessed and not the entire gene sequence will mean that other potentially important variants are missed, variants that may impact on clinical care. For example the currently FDA approved *UGT1A1* test does not include the reduced activity *6 allele in the coding region (211G>A, G71R), which is less common than *28 but has been shown to be predictive of hyperbilirubinemia, especially in Asians (4).

Second, an inexpensive biomarker, total serum bilirubin, is available as a clinical predictor of liver function, and can serve as a surrogate marker of UGT1A1 enzyme function and severe neutropenia prior to administering irinotecan (6). Some oncologists argue against the use of the *UGT1A1* genotype because they think bilirubin levels, with which they are more comfortable, may be as reliable an indicator as the genotype in predicting the appropriate selection of irinotecan dose (anecdotal evidence). However, there are no studies showing how total bilirubin levels may be used to guide irinotecan dose modification or *a priori* selection. Thus, a second challenge is uncertainty over the clinical utility of *UGT1A1* genotype and serum bilirubin in guiding the appropriate dose selection for irinotecan.

Third, there is no model for the delivery and interpretation of the *UGT1A1* test. In considering the FDA label change for irinotecan, the FDA recommends a reduced initial dose of irinotecan for patients homozygous for the *28 allele. The FDA recomendations go on to state however that the precise dose reduction in patients homozygous for the *28 allele is unknown and subsequent dose modifications should be tailored according to the individual patient's treatment tolerance (7). Unlike other genetic tests for disease prognosis, the reports for pharmacogenetic tests are currently not interpreted for the ordering physicians. There is neither a detailed explanation of how and to what extent the initial dose should be reduced, nor for how long the patient should remain on the reduced dose. The onus is on the ordering oncologist to consult with other colleagues and the literature to make sense of how to use the test result to manage irinotecan dosing. Without clear instructions on how to interpret and use the test results, oncologists, who are generally not trained in interpreting genetic tests, may be reluctant to order the test in the first place.

Discussion

Given the challenges for the appropriate use of the *UGT1A1* pharmacogenetic test, it is clear that a lot more needs to be done to clarify the role of the *UGT1A1* test in the management of irinotecan toxicity. Importantly, these challenges are not unique to the *UGT1A1* test and therefore solutions to redress knowledge gaps in the use of *UGT1A1* testing can extend to other pharmacogenetic tests. First, pharmacogenetic tests could be more clinically applicable if they included a comprehensive survey of variation in the human genome. Many current pharmacogenetic tests evaluate one or a few candidate genes with a biologically plausible link to drug responses. Although successful for some drugs, this approach may miss important contributions of variation in other genes, therefore reducing the predictive value of the test. It is promising that many recent pharmacogenomic studies employ a genome-wide approach. In addition, many populations such as African-Americans and Hispanics are currently under-represented in pharmacogenomic studies and potentially important variants in those groups are not well known. Although diversity in a study population may

complicate analysis, multiple pharmacogenomic studies in different ethnic groups will support the clinical use of the test in broader populations.

Second, it is essential to evaluate the clinical utility of pharmacogenetic testing, including the risks, costs and benefits relative to use of the available alternative biomarkers. Lack of clinical utility data is one of the reasons the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group cannot recommend for or against the clinical use of some pharmacogenetic tests (9). Such data would be very useful if third party payers are to shoulder the cost of pharmacogenomic testing.

Third, successful integration of pharmacogenetic testing in the clinic requires not only a clinical laboratory that establishes and validates the assay but also a rapid reporting system that provides appropriate guidance as to the interpretation of the test results. Given the complexity of the process, it will likely require the coordinated efforts of multiple healthcare professionals including laboratory medicine specialists, physicians, nurses, and pharmacists.

Finally, clinicians' knowledge of pharmacogenetic testing may also influence its successful integration into the clinic. A recent systematic review suggests that clinicians are not generally confident in providing genetic services to patients because of lack of training and knowledge (10). Therefore, efforts are needed to improve clinicians' knowledge of pharmacogenetic tests in order to facilitate their successful integration into clinical practice.

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Table

Selected pharmacogenetic tests available for clinical application.

Test[a]	Pharmacogenetic marker	Drug example	Disease example	Test example
Required[a]	*EGFR* expression[b]	Cetuximab	Colorectal cancer	EGFR pharmDx™
	HER2/NEU overexpression[b]	Trastuzumab	Breast cancer	Herceptest™
	CCR-5-tropic HIV-1[b]	Maraviroc	HIV infection	Trofile™
	Presence of Philadelphia chromosome[b]	Dasatinib	Acute lymphoblastic leukemia	BCR/ABL test
Recommended[a]	*HLA-B*1502*	Carbamazepine	Epilepsy	HLA typing
	*HLA-B*5701*	Abacavir	HIV infection	HLA typing
	CYP2C9 variants	Warfarin	Thromoboembolism	Verigene® Warfarin
	VKORC1 variants			Metabolism Nucleic Acid Test
	TPMT variants	Azathioprine, 6-MP, Thioguanine	Acute lymphocytic leukemia	Prometheus Therapeutics & Diagnostics and others[c]
	TPMT variants	Azathioprine, 6-MP, Thioguanine	Acute lymphocytic leukemia	Prometheus Therapeutics & Diagnostics and others[c]
	UGT1A1 variants	Irinotecan	Colorectal cancer	Invader® UGT1A1 Molecular Assay
	G6PD deficiency[b]	Rasburicase	Hyperuricemia	Glucose-6-phosphate dehydrogenase screening
Information only[a]	*c-KIT* expression[b]	Imatinib	Gastrointestinal stromal tumor	Dako Cytomation c-Kit pharmDx™
	CYP2C19 variants	Voriconazole	Fungal infection	Roche AmpliChip™ CYP450
	CYP2D6 variants	Atomoxetine, Tamoxifen, Voriconazole	Attention-deficit hyperactivity disease	Roche AmpliChip™ CYP450
	DPD deficiency[b]	Capecitabine, 5-FU	Colorectal cancer	Genelex and others
	EGFR expression[b]	Erlotinib	Non-small cell lung cancer	EGFR pharmDx™
	G6PD deficiency[b]	Primaquine	Malaria	Glucose-6-phosphate dehydrogenase screening
	NAT variants	Isoniazid, Rifampin	Tuberculosis	Genelex and others[c]
	Absence of Philadelphia	Busulfan	Chronic myelogenous leukemia	BCR/ABL test
	UGT1A1 variants	Nilotinib	Chronic myelogenous leukemia	Invader® UGT1A1 Molecular Assay

Abbreviations: *EGFR* = Epidermal growth factor receptor; *HER2/NEU* = v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; *CCR-5* = Chemokine C-C motif receptor; *HIV*=human immunodeficiency virus; *BCR* = breakpoint cluster region; *ABL* = Abelson; *HLA* = Human leukocyte antigen; *CYP2C9* = Cytochrome P450 2C9; *VKORC1* = Vitamin K epoxide reductase complex subunit 1; *CYP2D6* = Cytochrome P450 2D6, *TPMT* = Thiopurine S-methyltransferase; *UGT1A1* = Uridine diphosphate glucuronosyltransferase 1A1; *c-KIT* = v-kit Hardy-Zuckerman 4 feline sarcoma viral

oncogene homolog; *CYP2C19* = Cytochrome P450 2C19; DPD deficiency = dihydropyrimidine dehydrogenase; G6PD = glucose-6-phosphate dehydrogenase; *NAT* = N-acetyltransferase; *PML/PAR* = Retinoic acid receptor; PCR = polymerase chain reaction; 6-MP = 6-mercaptopurine; 5-FU: 5-fluorouracil.

[a] Classified by the Food and Drug Administration

[b] These tests determine whether a particular gene is expressed. The other tests determine the sequence variability of a particular gene.

[c] A home-brew test offered by a commercial clinical laboratory

[*] For patients with an Asian ancestry