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Important preparatory steps and clinical considerations for pharmacogenetics adoption into practice

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Abstract

Genetic influence on medication response has been well documented; yet, the clinical integration of pharmacogenetics has been slow. Lack of knowledge on the fundamentals of pharmacogenetic testing among clinicians and the complexities surrounding test selection, results interpretation, and clinical utility, among others, contribute to this slow adoption. In this paper, we describe integration of pharmacogenetics in the clinic, selection of target population and testing laboratories, team of expertise needed, available resources, and critical considerations for accurate interpretation of test results. We also discuss phenoconversion and pharmacogenetic utility in special populations including pediatrics, pregnant women and transplant patients. Ultimately, it should be observed that pharmacogenetics is an additional piece in the prescribers' toolbox to guide prescribing decisions. Other intrinsic and extrinsic factors should be considered for more accurate and personalized prescribing practice. This paper aims to guide clinicians with preparatory steps and "clinical pearls" necessary to successfully integrate pharmacogenetics into routine practice.



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Keywords: Pharmacogenomics, pharmacogenetic testing, personalized medicine, precision medicine, phenoconversion, pharmacogenetics in special populations, pharmacogenetics

INTRODUCTION

Medical professionals have long observed that some individuals respond differently to treatments than expected. Standard therapeutic doses have been proven to be ineffective and even harmful for some individuals^[1,2]. Such interpatient variability can be attributed to a wide host of factors, including the genetic makeup of the individual^[3]. Pharmacogenetics - the study of how genes influence one's response to medications - is an application of personalized medicine with clinically actionable examples^[4]. Elements necessary for clinical implementation of pharmacogenetics such as robust and consistently replicated scientific evidence, affordable and high-throughput testing technologies, electronic health records (EHR)-enabled clinical decision support (CDS), and expert-driven resources for pharmacogenetics-guided therapeutic recommendations, are all available and in use at some academic and non-academic institutions^[4,5]. However, its widespread adoption is still lagging behind. Efforts from Pharmacogenomic Knowledgebase (PharmGKB) and professional societies such as the Clinical Pharmacogenetic Implementation Consortium (CPIC) and the Dutch Pharmacogenetic Working Group (DPWG) are actively ongoing and helping to overcome the challenge related to evidence and clinical guidance^[4,6]. Early adopter and implementation programs in collaboration with federal networks/consortia are also working to address the remaining challenges and provide process best-practices for this discipline^[7]. For instance, patient education materials and protocol resources have been developed by the Clinical Sequencing Evidence-Generating Research (CSER) (<https://cser-consortium.org/>). The Electronic Medical Records and Genomics (eMERGE) Consortium investigators developed validated electronic phenotyping algorithms named Phenotype Knowledge Base (<https://phekb.org/>) and also curated member-sites' implemented clinical decision support rules on their Clinical Decision Support Knowledge Base (<https://cdskb.org/>). In addition, the Implementing Genomics in Practice (IGNITE) Network has developed the Spark Toolbox (<https://gmkb.org/ignite-toolbox-overview/>) which hosts educational and implementation resources for researchers and clinicians.

Most clinicians agree that genetics impact response to medications^[8], and are favorable towards pharmacogenetics in general^[9]; however, their lack of appreciable knowledge of pharmacogenetic utility in practice have led to some resistance and slow uptake^[9].

The purpose of this manuscript is to provide stakeholders, particularly clinicians, who are interested in pharmacogenetic implementation with pre-implementation preparatory steps and other considerations for effective clinical adoption. We also discuss available pharmacogenetic resources, how to select genes and laboratories for testing and how to interpret and utilize the results to guide prescribing decisions [Table 1]. This manuscript is intended to provide a broad overview of pertinent points to consider prior to embarking on integration of this discipline into routine practice. Several actionable pharmacogenetic drug-gene pairs associated with psychiatry, pain, cardiology, and others are highlighted throughout the paper as examples for the various discussion points.

SELECTING THE MEDICATIONS, GENES, AND THE LABORATORY FOR TESTING

The medications

As of August 2020, there are 24 CPIC guidelines covering approximately 46 medications from cardiology to pain, infectious diseases, oncology, psychiatry, and others. A full list of the guidelines can be accessed via the CPIC website (<https://cpicpgx.org/guidelines/>). The United States Food and Drug administration (FDA) has also included pharmacogenetic information in the drug labeling for 279 drugs^[10]. This information is

Table 1. Key preparatory steps in establishing pharmacogenetic implementation programs

Preparatory steps	Elements	Points to consider
Selecting the medications, genes and the laboratory	The medications	<ul style="list-style-type: none"> Resources available include CPIC, FDA Table for Pharmacogenomic Associations, DPWG Guidelines, FDA Tables of Pharmacogenetic Biomarkers, etc. Clinically actionable examples can be found in many clinical specialties such as cardiology, pain, psychiatry, infectious diseases, oncology, gastroenterology, transplantation, neurology, and others Some high-risk PGx medications are backed by randomized controlled trials, e.g., Abacavir, carbamazepine, and mercaptopurine Some high-risk PGx medications have black box warnings highlighting their PGx associations, e.g., warfarin and clopidogrel
	The genes	<ul style="list-style-type: none"> Gene selection is mainly driven by medications in a reactive testing model Of note, the 24 CPIC guidelines cover 19 genes Most early adopters initiate their programs with the CYP genes, e.g., <i>CYP2C19</i>, <i>CYP2D6</i>, and <i>CYP2C9</i> Other commonly implemented genes include <i>VKORC1</i>, <i>SLCO1B1</i>, and <i>HLA-B</i> Pre-emptive testing approach with a panel that covers common genes may reduce the need to choose which gene(s) to test first Genes to be tested must have documented implications for medication response, evidence of association with medication-related outcomes and pharmacogenetic-guided therapeutic recommendation All relevant variants must be reviewed and interrogated during testing
	The test and the lab	<ul style="list-style-type: none"> Laboratory must be CAP/CLIA accredited Test can be conducted in-house or with a commercial laboratory Commercial lab reports usually contains information on genes and variants tested, genotypes, and phenotypes (<i>pre-2019 reports may have included high-risk medications and PGx-guided therapeutic recommendations</i>)
Selecting the patients to test	The patients	<ul style="list-style-type: none"> Consider patients who have experienced adverse drug events or failed therapies in the past. Consider patients who are diagnosed with indications for which a PGx medication treatment is anticipated. Consider patients who meet third party coverage determinations.
Providing oversight for implementation program	The multidisciplinary team	<ul style="list-style-type: none"> Success of this program is the responsibility of a multidisciplinary team Pharmacists are leaders in this discipline and they play many key roles Other team members include physicians, geneticists, laboratory personnel, informaticians, IT experts, nurses, patient advocates, institutional leadership, etc.

CPIC: Clinical Pharmacogenetics Implementation Consortium; FDA: United States Food and Drug Administration; DPWG: Dutch Pharmacogenetics Working Group; PGx: pharmacogenetics; CYP: cytochrome P450; CAP/CLIA: College of American Pathologists/Clinical Laboratory Improvement Amendments

classified as informative, actionable, and testing recommended or required. In February 2020, the FDA published a table for pharmacogenomic associations which further delineates the type of pharmacogenetic association as it relates to efficacy, adverse events, and pharmacokinetic associations^[10]. There are some overlap between CPIC guidelines and the FDA's table for pharmacogenomic associations; however several differences have been noted as well. To date, there are 41 medications that have both CPIC guidelines and FDA pharmacogenetic information. Of these 41 medications, the FDA drug-labels recommend pharmacogenetic testing for four (i.e., oxcarbazepine, azathioprine, mercaptopurine, and thioguanine), and requires testing for four more medications (i.e., abacavir, carbamazepine, ivacaftor, and rasburicase). Specifically, abacavir, carbamazepine, and mercaptopurine are supported by randomized controlled trials that documented improved clinical outcomes and cost effectiveness of pharmacogenetic testing before the initiation of these medications^[11-13]. The FDA has also updated some drug labels with black box warnings for their pharmacogenetic associations such as clopidogrel, codeine, rasburicase, and pegloticase (<https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling>) [Table 2].

The genes

Pharmacogenetic testing is the process of interrogating the implicated genes and variants to determine one's genotype and corresponding clinical phenotypes. The selection of the right gene(s) to test depends on the medication(s) of interest. The two dozen CPIC guidelines published to date cover 19 genes. Most early adopters of pharmacogenetics initiate their programs with the genes that encode the cytochrome *P450* (*CYP*)

Table 2. Drug-Gene pairs with strong FDA recommendations and the commercial laboratories that offer CLIA-certified clinical pharmacogenetic tests in the U.S.

Drug	Gene(s)	US FDA PGx designation	Commercial labs that offer testing [#]
Clopidogrel	<i>CYP2C19</i>	Boxed warning	Fulgent Genetics; Sema4; Admera Health; RPRD Diagnostics, LLC; OneOme LLC; Sanford Medical Genetics Laboratory Sanford Imagenetics; Color; Pathway Genomics
Codeine	<i>CYP2D6</i>	Boxed warning	Fulgent Genetics; Sema4; OneOme LLC; Admera Health; RPRD Diagnostics LLC; Sanford Medical Genetics Laboratory Sanford Imagenetics; Color; Pathway Genomics
Tramadol	<i>CYP2D6</i>	Boxed warning	Fulgent Genetics; Sema4; OneOme LLC; Admera Health; RPRD Diagnostics LLC; Sanford Medical Genetics Laboratory Sanford Imagenetics; Color; Pathway Genomics
Pegloticase	<i>G6PD</i>	Boxed warning/testing required*	EGL Genetic Diagnostics Eurofins Clinical Diagnostics; Invitae; Fulgent Genetics; Baby Genes by ArcherDX Clinical Services; Sema4; RPRD Diagnostics, LLC
Rasburicase	<i>G6PD</i>	Boxed warning/testing required*	EGL Genetic Diagnostics Eurofins Clinical Diagnostics; Invitae; Fulgent Genetics; Baby Genes by ArcherDX Clinical Services; Sema4; RPRD Diagnostics, LLC
Carbamazepine	<i>HLA-B*1502</i>	Boxed warning/testing required*	OneOme, LLC; Admera Health; RPRD Diagnostics, LLC; Pathway Genomics
Oxcarbazepine	<i>HLA-B*1502</i>	Testing required*	OneOme, LLC; Admera Health; RPRD Diagnostics, LLC; Pathway Genomics
Abacavir	<i>HLA-B*5701</i>	Boxed warning/testing required*	OneOme, LLC; Admera Health; RPRD Diagnostics, LLC; Pathway Genomics
Azathioprine	<i>TPMT/NUDT15</i>	Testing recommended	RPRD Diagnostics, LLC; Admera Health; OneOme, LLC; Color
Mercaptopurine	<i>TPMT/NUDT15</i>	Testing Recommended	RPRD Diagnostics, LLC; Admera Health; OneOme, LLC; Color
Thioguanine	<i>TPMT/NUDT15</i>	Testing recommended	RPRD Diagnostics, LLC; Admera Health; OneOme, LLC; Color

Testing required means patients should be screened prior to initiation of therapy; Testing required* means genetically at-risk populations based on ancestries should be tested prior to initiation of therapy; Testing recommended means patients who experience adverse drug reactions should be tested; [#]not a comprehensive list. All commercial labs listed offers CLIA-certified, state-licensed, saliva and peripheral (whole) blood specimen options, U.S.-based, clinical tests. Only laboratories indexed on Genetic Testing Registry (<https://www.ncbi.nlm.nih.gov/gtr/>) were considered for this table; CLIA - Clinical Laboratory Improvement Amendments

enzymes responsible for the majority of drug metabolism - namely *CYP2C9*, *CYP2C19*, and *CYP2D6*. These three enzymes collectively metabolize about 40% of all medications^[14]. Ultimately, the choice of the genes to test and when to test should be based on the nature of the practice site (pre-emptive or reactive), the clinical setting (e.g., cardiology vs. pain vs. psychiatry), and the target patient populations. In preemptive testing, a testing panel that covers the common “pharmacogenes” may be considered so that information resulted will be applicable to future prescribing decisions. In reactive testing, the medication prescribed will determine the gene to be tested. Moreover, when initiating a targeted, clinical specialty-focus program (e.g., cardiology pharmacogenetics clinic), a careful review should be conducted to use panels that target genes implicated in the response to cardiovascular medications, have evidence of genetic association with medication-related outcomes, and pharmacogenetic-guided therapeutic recommendation such as clopidogrel, warfarin, and simvastatin.

Once the genes are selected for testing, a thorough review should be conducted to ensure that the test will interrogate all the relevant and actionable variants based on evidence and target population^[15]. For instance, if your clinic or institution serves African ancestry patients, the *5, *6, *8 and *11 variant alleles should be included in the test for the *CYP2C9* gene along with the common *2 and *3 alleles. Moreover, clinicians should note that the designation of *1/*1 or normal metabolizer is simply the absence of the set of interrogated variant alleles in the tested patient. Clinicians should ensure that the selected tests, whether single-gene or panel-bases: (1) cover genes that are relevant for the medications in question; and (2) interrogate a list of diverse variants in those genes that will inform therapeutic decisions for the target patient population. The Association for Molecular Pathology (AMP) advises on the minimum set of variant alleles for each gene, which is an excellent guide for those charged with selecting a pharmacogenetic test. To date, the AMP has published guidance for *CYP2C19*^[16] and *CYP2C9*^[17], and this group is planning to publish similar guidance for *CYP2D6* and other “pharmacogenes”.

The test and the laboratory

Many commercial laboratories have added pharmacogenetics to their test offerings. Traditionally, these laboratory-developed tests have been under the supervision of the U.S. FDA; however, in August 2020, the U.S. Department of Health and Human Services issued a statement that the FDA will not require pre-

market review of laboratory-developed tests. Instead, they can still regulate these tests through the longer and more time consuming “notice and comment rulemaking process.” This is an evolving topic and more on this regulation is anticipated^[18]. These tests predominantly are only required to prove analytical validity, and the genes and variants being tested are neither regulated nor standardized. This could be problematic for accurate phenotype calling necessary for selecting the appropriate therapeutic guidance^[15]. Additionally, some of the commercial laboratories may test and report on genes and variants that have questionable clinical utility based on limited evidence^[19]. A threshold of evidence for reporting and actionability of the returned genotypes should be pre-defined as guided by FDA, CPIC, DPWG, and PharmGKB^[15]. Additional drug-gene pairs on the panel with low evidence not meeting the threshold set by the implementation team should not be implemented. Rather, such information should be stored for future use when strength of evidence rises to actionability thresholds such as update of FDA drug labeling to include testing required, testing recommended, alternate medication or dosing changes, and/or CPIC Level A evidence designation with or without a published guideline. Implementation team members should pay careful attention to pharmacogenetics literature to track and react in a timely manner to new and updated information.

Notably, the *CYP2D6* is an example of how the variability and lack of standardization across commercial labs could be demonstrated. *CYP2D6* is a highly polymorphic gene with complexity demonstrated in copy number variation, gene deletion, hybrid alleles, with varying allele frequency for the variation across various ancestral populations. Variability in the method of *CYP2D6* genotyping can inaccurately report the *CYP2D6* phenotype if the genetic variation that is predominantly frequent in the health system is not captured, leading to erroneous prescribing actions. Several papers have instructed on the selection of genes and minimum number of variant alleles necessary for capturing an accurate phenotype^[15,20]. When implementing *CYP2D6*, implementers should confirm from laboratory officials, in-house or commercial, whether: (1) the test interrogates *CYP2D6* alleles prevalent in the relevant patient populations; and (2) the test can detect with accuracy when haplotypes are duplicated, and if so, which haplotype/(s) is/are duplicated. Lastly, implementing clinicians should be completely aware of how the laboratory translates genotypes into phenotypes. Ideally, both the genotypes of assayed variants and phenotypes should be provided to the clinician for verification and clinical interpretation.

Pharmacogenetic testing can be offered through a commercial or in-house laboratories provided they are accredited or certified by the College of American Pathologists (CAP) and the Clinical Laboratory Improvement Amendments (CLIA)^[21]. Furthermore, most commercial pharmacogenetic tests are usually accompanied with a personalized report that details the variants tested for each gene, genotypes, phenotypes, and before 2019, the implicated medications along with genotype-guided therapeutic recommendations. In April 2019, the FDA issued a warning letter to Inova for marketing their pharmacogenetic tests with claims that it can predict patients’ response to medications^[22]. Since then, many commercial laboratories report results without a reference to the implicated medications or pharmacogenetic-guided therapeutic recommendations^[23]. This development may negatively impact pharmacogenetics adoption into clinical practice and further embolden the resistance to implementation especially for clinicians who rely on the commercial laboratory reports’ guidance for their therapeutic decisions. The FDA’s concern that “providers may make inappropriate treatment decisions” using these reports as some laboratories report on drug-gene pairs with insufficient evidence to warrant clinical utility. However, a complete omission of genetic-guided therapeutic recommendations from these reports is not the solution. Report content should be standardized in a joint effort between relevant stakeholders such as pharmacogenetic experts (i.e., researchers and clinical implementers), laboratory officials, and authorities including the FDA. Until then, this development presents a unique opportunity for pharmacogenetics-trained pharmacists to bridge the knowledge gap. They can help prescribers decipher the clinical implications of the genetic results as well as recommend genetic-guided therapeutic alternatives and/or dose optimizations for patients.

SELECTING THE PATIENTS TO TEST

Target patient populations

The question of “who to test” still remains an important and often uncertain one. Some may argue that choosing patients who have experienced adverse events, failed therapy, or simply focusing on “high risk patients” may be a reasonable approach^[24]. Others suggest selecting patients who have an indication for a procedure for which a prescription for pharmacogenetic medication(s) can be anticipated^[24]. Elective percutaneous coronary intervention (PCI) is an example of such procedures as patients are likely to be started on antiplatelet therapy for which *CYP2C19* genotyping is important. Current data suggest that in many instances, patients who are genotyped for a certain indication such as PCI or with initiation of new medications are often prescribed other medications for which the pharmacogenetic test may also be relevant^[25]. Early implementors have focused on patients who are expected to be prescribed medication with pharmacogenetic evidence such as the RIGHT patients in the Mayo Clinic and the PREDICT population as summarized by Dunnenberger *et al.*^[26].

Early adopters of genomic medicine and pharmacogenetics envisioned a time where genetic information will be generated on every patient, to be used along other data to individualize therapy. While this will be ideal to realize the widespread promise of precision medicine, barriers such as lack of institutional resources, cost of testing, lack of insurance coverage, and lack of infrastructure to support the use of genomic information pose a challenge to the “Genotype Everyone” approach^[27]. Thus, selecting a candidate patient population to either justify payer’s coverage of a pharmacogenetic test or build evidence for the utility of the data is still an approach most commonly taken by many institutions. Research investigators and implementers continue to collect data on patient outcomes, cost effectiveness, and the clinical utility of pharmacogenetic testing.

Some institutions initiated with a single drug-gene pair indicated by a clinical procedure or indication before expanding into other drug-gene pairs in the ambulatory setting^[28,29]. Others start with medications with robust evidence for serious adverse events (such as thiopurine methyltransferase and thiopurines) before evolving into a preemptive testing approach^[30]. Some institutions have also utilized algorithms to predict and prioritize those who would benefit the most from testing. For example, enterprise-level pharmacogenetic testing was performed by the Mayo Clinic and Vanderbilt University, both of which use a preemptive testing approach^[31,32]. Through the RIGHT protocol at Mayo Clinic, a multivariate prediction algorithm identifies patients with high cardiovascular risk profile who are likely to be prescribed multiple medications with pharmacogenetic evidence, and therefore benefit from preemptive testing, was used to recruit 1000 patients^[31,33]. INGENIOUS Trial at Indiana University uses a multi-gene panel to test patients who receive a first prescription for at least one of 27 medications with pharmacogenetic guidelines^[34].

Where resources are limited, clinicians may adopt the model that prioritizes patients likely to be prescribed multiple pharmacogenetic medications (e.g., medications with CPIC guidance) as their initial roll out. A recent study investigating the utilization of pharmacogenetic medications using commercial claims of more than 100,000 patients with PCI, documented that 50% and 68% of patients were prescribed one or more drugs with pharmacogenetic evidence (CPIC level 1A or 1B) in addition to the antiplatelet therapy, over one and five years, respectively. This data suggest that a multi-gene panel testing may have a high utility in patients with PCI as these patients are frequently prescribed multiple medications with high pharmacogenetic evidence^[25]. And therefore, PCI patients who are genotyped for *CYP2C19* for antiplatelet selection may be good targets for additional gene testing to guide other commonly prescribed medications in this high-risk cardiac patient population. Furthermore, a United States Department of Veterans Affairs (VA) study recently demonstrated that among over seven million veterans, 55% were prescribed one or more CPIC level medications, with 15% and 11% being prescribed two and three of such medications, respectively^[35]. The study projected that 92%, 26%, and 4% of veterans could have been prescribed warfarin,

simvastatin, and tramadol, respectively; while carrying actionable variants in *CYP2C9* or *VKORC1*, *SLCO1B1*, *CYP2D6*. These data collectively suggest that new practice sites should investigate the utilization and prevalence of medications with pharmacogenetic implications and prioritize testing patients who are commonly prescribed such medications.

Investigator-initiated studies and NIH funded pragmatic clinical trials are underway to prospectively assess clinical utility and cost effectiveness of pharmacogenetic implementation, and potentially will shed light on strategies for selecting patients. For example, the National Human Genome Research Institute (NHGRI) has recently funded two pragmatic trials named “ADOPT PGx” to investigate genotype-guided approaches for prescribing opioids and antidepressants. In addition, the ongoing Ubiquitous Pharmacogenomics (U-PGx) study in Europe plans to investigate the outcomes of preemptive pharmacogenetic testing in seven European countries^[36]. Until these prospective data become available, it seems reasonable to focus implementation efforts on patients who are likely to be prescribed multiple medications with pharmacogenetic evidence, particularly those who anticipate a clinical procedure or initiation of a pharmacogenetic medication. Also, patients who have access to and can afford the test or have insurance coverage can be targeted to generate initial data to support widespread implementation. It should be noted that some commercial laboratories offer income-based payment assistance programs which provides about 50%-95% discount to qualifying patients in an effort to address disparities^[37]. In the absence of such assistance, efforts should be made to encourage institutional pilot funding for patients who are unable to afford the test. Perhaps, there should also be federal grant funding allocated specifically for pharmacogenetic implementations in low-income populations. Moreover, it is logical that a multi-gene panel that test for genes and variants relevant for the medications commonly prescribed in this patient population, may potentially translate into a high economic value investment for the institution and third party-payers. Van Driest *et al.*^[32] observed that 91% of genotyped patients and 96% of African Americans carry at least one actionable variant when genotyped for five genes namely *CYP2C19*, *CYP2C9*, *VKORC1*, *thiopurine methyltransferase (TPMT)*, and *CYP3A5*. It is important to estimate the prevalence of actionable genotype variants in the health system, which largely depends on the ancestral distribution of the patients. Implementers have either used genotype data generated for their population^[32,38] or projected the rate of actionable genotypes using published population frequency from CPIC and 1000 Genomes, among others^[35]. Of note, the frequency of actionable phenotypes may be higher if concomitant administration of enzyme (i.e., *CYP2D6*, *CYP2C19*, *etc.*) inhibitors is considered. Mostafa *et al.*^[39] recently documented that the prevalence *CYP2D6* and *CYP2C19* poor metabolizer phenotypes were increased by fivefold after considering co-medication with substrates that can inhibit these enzymes among 5,408 Australians. More on enzyme inhibitors is discussed below in the phenoconversion section.

Surveys are valuable to understanding implementation challenges and work out solutions to overcome encountered barriers. A recent paper that surveyed the physicians within a pharmacogenetic implementation program reported that having genotype data in the EHR before a patient-physician encounter is critical for effective implementation^[28]. This suggests focusing on patients who are anticipated to have pre-appointment visits where patients can be genotyped, and information is returned to EHR before a physician encounter. In addition, an approach that targets patients scheduled for elective arthroplasty for *CYP2D6*-guided opioid prescribing may prove to be successful. Patients in this setting can be genotyped at a pre-operative visit with the results returned in the EHR for real-time use and effective genotype-guided prescribing^[40]. Within the primary care setting, patients who are predicted to start a medication with pharmacogenetic recommendations may also be targeted for pharmacogenetic testing. If the genotype cannot be made available before the patient-physician encounter, discussions should be made around the willingness of physicians and patients to wait for the genotyping results before prescribing the medication. This was the case in a recent pragmatic clinical trial of *CYP2C19* implementation for proton pump inhibitor use in pediatrics, where patients and physicians were willing to wait on the genotyping results for genotype-

guided proton pump inhibitor prescribing^[41]. Although patients can be started on a medication before the return of genotyping results, this is not an ideal model, and may result in patients being lost to follow up with increases in economic burden for patients and healthcare system due to the costs of treatment for adverse drug reactions, treatment failures and even the cost of the wasted initially filled medication.

PROVIDING OVERSIGHT FOR IMPLEMENTATION PROGRAM

The multidisciplinary implementation team

Several academic and non-academic institutions have spearheaded pharmacogenetic implementation efforts, documenting site-specific implementation experiences and lessons learned. These implementation efforts were summarized in other review papers^[26,42]. Many of the implementation services were pharmacist-led^[43-45]. Pharmacists play many key roles in the implementation of pharmacogenetics such as reviewing medication use and safety policies, developing educational strategies for other providers and patients, reviewing and analyzing evidence, managing the pharmacogenetic database, and implementing overall leadership^[43]. Physicians are also critical to the development and success of the implementation. Understanding their interest within a practice site and how they perceive pharmacogenetics in their clinical workflow is important for successful integration. Structured or semi-structured surveys administered at the pre-implementation stage may help implementing clinicians glean understanding of the institutional landscape, stakeholders' goals, and clinicians' interest and their perspectives about the use of pharmacogenetics^[46]. Physician-champions should be identified and in the planning process for the targeted clinical group and/or specialty. Their clinical expertise is vital especially when making protocol decisions on pharmacogenetic-guided therapeutic recommendations and their associated alternative therapies or dosing. Like pharmacists, physicians should be included in the oversight committee that is established early on to oversee the activities from the planning phase through implementation of clinical pharmacogenetics and post-implementation evaluation. Already established Pharmacy and Therapeutics (P&T) Committees can serve this purpose as well. Additionally, experts from genetics, clinical laboratory, informatics and information technology, administration, institutional leadership and patient advocacy should be included in this committee.

RELEVANT RESOURCES FOR PHARMACOGENETIC IMPLEMENTATION

Clinical Pharmacogenetics Implementation Consortium

The CPIC is an international consortium of volunteers and dedicated members who are interested in facilitating the use of pharmacogenetic information for patient care. It was created in 2009 as a shared project between the international pharmacogenetics database and website, PharmGKB (www.pharmgkb.org) and the NIH. The purpose of CPIC is to provide freely available, peer-reviewed, evidence-based clinical pharmacogenetic practice guidelines. As of 2019, CPIC has a growing membership of 350 members from 245 institutions in 33 countries with observers from the U.S. FDA and NIH. Members meet monthly on general membership calls and through the informatics, dissemination, and other workgroup meetings. CPIC guidelines are indexed in PubMed and published on the CPIC website (<https://cpicpgx.org/guidelines/>) as well as annotated on the PharmGKB website (www.pharmgkb.org). As of August 2020, there are 24 published guidelines with 2 more in progress. Their goal is to update the guidelines on a biennial schedule. So far, they cover 19 genes and 46 medications across several therapeutic areas including infectious diseases, cardiology, psychiatry, neurology, and oncology. CPIC also compiles a list of contacts for PGx implementers across the U.S. (<https://cpicpgx.org/implementation/>) and other countries, which is a great resource of contacts for experts and clinicians in the field.

United States Food and Drug Administration

Through their oversight of drug product labeling, the FDA also maintains a table of pharmacogenomic biomarkers (<https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers->

drug-labeling). In February 2020, they published a table of pharmacogenetic associations (<https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations>) to highlight drug-gene associations that are likely to have altered metabolism and, in certain cases, differential therapeutic effects such as risks for adverse events and therapeutic failures. This is a living document and the FDA has an open docket for stakeholders to offer specific comments about inclusion of other pharmacogenetic associations along with the rationale and supportive evidence. It is unclear at this point how often this table will be updated. While CPIC has published their process for robust and systematic evidence review to develop their peer-reviewed guidelines^[47]; the FDA does not explicitly discuss the evidence base underlying the recommendations presented in their table of pharmacogenetic associations.

Dutch Pharmacogenetics Working Group

In 2005, the Royal Dutch Pharmacists Association established the DPWG with the objective of developing pharmacogenomic-based therapeutic and dose recommendations. Thus far, recommendations have been developed for over 80 drugs covering various therapeutic classes covered in both the CPIC and FDA databases. These guidelines are updated every 3 months and are available to all physicians and pharmacists in the Netherlands. They have also been integrated into the clinical decision support systems in the Netherlands and serve as the main source of pharmacogenetic-guided recommendations used in U-PGx in Europe^[6,36].

Of note, the FDA, CPIC, and DPWG guidelines offer similar recommendations for most drug-gene pairs; however, differences in the guidelines' development processes and/or allele and phenotype classifications have resulted in discordance in some recommendations^[48]. For instance, pharmacogenetic-guided recommendations for aripiprazole, clozapine, and gefitinib, among others, vary across all three platforms. CPIC and DPWG are currently working on standardization of alleles and phenotypes^[49]. The recent *CYP2D6* genotype-phenotype term standardization project is one of the most important efforts in this area^[50]. Using standardized terms and harmonizing the guidelines are likely to facilitate clinical implementation efforts and data sharing across health systems around the world.

The Canadian Pharmacogenomics Network for Drug Safety

The Canadian Pharmacogenomics Network for Drug Safety (CPNDS) is an innovative, national program that aims to reduce serious adverse drug reactions in children and adults^[51]. They have developed their own specific "drug biotransformation" testing panel which contains variants of genes that are known to influence the way a patient responds to a given medication or may predispose a patient to an adverse drug reaction. Having a national health care system in Canada with an active surveillance network facilitates the integration and dissemination of CPNDS guidelines to accelerate adoption of pharmacogenetics.

OTHER PERTINENT CLINICAL CONSIDERATIONS

Assessment of pharmacogenetic results for clinical applications should also account for non-genetic clinical and environmental factors to evade incomplete conclusions and misguided recommendations. Drug-drug interactions, disease states, organ function, hormonal status, and considerations of additional patient specific factors are regularly taken into consideration for medication and dose selection. Drug-drug-gene interactions should be duly considered as the efficacy and incidence of adverse events are chiefly the result of parent compound and metabolite concentrations. In this section, we will review the concept of phenoconversion, considerations for switching medications, and guidance for special populations including transplant and pediatric patients as well as pregnant women. Other disease states have also been found to modulate the activity of metabolizing enzymes, though with less clarity for clinical actionability and therefore will not be covered here^[52].

Selecting an alternative therapy based on pharmacogenetic results

As with any therapy selection, prior patient experience with the medication, adherence, and cost should be taken into consideration. Apart from the usual clinical considerations for prescribing such as age, organ function (i.e., kidney, liver, etc.), co-medications, and others, close attention should be paid to these three principles when selecting an alternative therapy based on pharmacogenetic results - (1) the indication for the medication; (2) pharmacogenetic implications for the alternative therapy; and (3) loading dose requirements. Some pharmacogenetic recommendations are indication specific because the evidence for clinical actionability has been consistently replicated in select patient populations. This is the case with clopidogrel and *CYP2C19* intermediate and poor metabolizers who have acute coronary syndrome and have had a PCI. Although clopidogrel has other indications, only alternative therapies that produce antiplatelet effects for post-PCI patients should be considered in this population (i.e., prasugrel or ticagrelor). Secondly, clinicians must also consider the pharmacogenetic influences with the alternative medication and ensure that patients do not carry high risk variants for the alternative agents. For example, palonosetron would not be an ideal alternative for *CYP2D6* ultrarapid metabolizers who are not responsive to ondansetron since it is also a substrate of the *CYP2D6* metabolic pathway. Of note, the CPIC guideline incorporates this consideration into their therapeutic recommendations. Lastly, clinicians must confirm whether loading doses should be administered when making the switch to an alternative medication. For instance, when switching from clopidogrel to prasugrel or ticagrelor within the first 30 days post-PCI, the use of 60-mg or 180-mg loading dose, respectively, has been shown to be beneficial. However, it is reasonable to omit the loading dose if switching beyond 30 days from PCI^[53]. Many of institutions who implemented pharmacogenetics rely on trained pharmacists to evaluate the pharmacogenetic results and recommend changes to the prescribing clinician. These recommendations are better implemented if they are communicated through consult notes with specific recommendations in concise format. Interventions are likely to happen if clinicians have a chance for a patient encounter after the pharmacogenetic results are reported in EHR.

Phenoconversion

Pharmacogenetic results can be applicable throughout a patient's lifetime depending on the variants interrogated and the genotype result. However, extrinsic factors such as concomitant medications can lead to a transient discordance between the genotype and its corresponding phenotype - this concept is termed as "phenoconversion" or "drug-drug-gene interaction"^[54]. Currently, evidence shows *CYP2D6*, *CYP2C19*, *CYP2C9*, and *TPMT* are enzymes susceptible to phenoconversion^[55]. These enzymes are subject to inhibition and induction (except for *CYP2D6* which is not inducible) by medications and thus concomitant medications administered for patients must be interrogated for phenoconversion. Phenoconversion is most commonly seen with patients carrying alleles encoding for functional enzymes. For example, genetic results interpreted as a *CYP2D6* normal metabolizer can phenotypically present as a *CYP2D6* poor metabolizer due to the presence of strong *CYP2D6* inhibitor (e.g., paroxetine or fluoxetine)^[56]. In general, poor and ultrarapid metabolizers (of *CYP2C19*, *CYP2C9*, and *CYP2D6*) are less likely to be affected by this phenomenon, but the clinical impact will depend on the co-administered drugs^[57]. Poor metabolizers carry two alleles that produce decreased or nonfunctional enzymes, thus strong inhibition and induction may not affect the functional activity of the enzyme. For ultrarapid metabolizers, or carriers of increased transcription alleles or gene duplication, the risk of phenoconversion is less likely but still possible with strong inhibitors and substrate medications highly dependent on the enzyme^[57,58]. Lastly, considerations of how dependent the substrate medication is on the enzyme pathway (e.g., major or minor pathway) will determine the relevance of the gene and therefore the likelihood of phenoconversion being clinically relevant. Alternative metabolic pathways, for patient encoding functional enzymes, will decrease the clinical impact of an inhibitor or inducer for patients. Clinically relevant strong inhibitors should be monitored when being prescribed with other substrates of the same enzyme pathway. In this section we will discuss common clinically actionable drug-drug-gene interactions examples with *CYP2D6*, *CYP2C19*, *CYP2C9*, and *TPMT*.

CYP2D6

For the highly polymorphic *CYP2D6* gene, calculation of an activity score is used to translate genetic variants to phenotype (e.g., metabolizer status)^[59]. The CPIC guidelines have addressed incorporation of an inhibitor drug when calculating activity scores based on a system used for tamoxifen in breast cancer patient study^[60,61]. Substrates of *CYP2D6* include propafenone, paroxetine, risperidone, codeine, tramadol, and atomoxetine^[62]. When concomitant strong *CYP2D6* inhibitors are present, the activity score is multiplied by zero and the resultant phenotype is a poor metabolizer while for moderate to weak inhibitors the activity score is halved, and so the implicated phenotype will vary. For example, paroxetine significantly inhibits flecainide, desipramine, aripiprazole, and R-methadone metabolism in individuals carrying two functional alleles than those with decreased function alleles^[55]. In addition, since herbal supplements are also metabolized via the *CYP450* enzyme system and can cause phenoconversion, patients should be cautioned to report all active herbal supplement usage. Supplements such as bush mint, bush tea, or pignut should be monitored for their inhibitory effects on *CYP1A2*, *CYP3A4*, and especially *CYP2D6*^[52].

CYP2C19

The interaction between proton pump inhibitors and clopidogrel is attributed to inhibition of *CYP2C19*, however this has not been found to be a class effect by all proton pump inhibitors. Their influence on clopidogrel bioactivation differs by the agent and by the *CYP2C19* metabolizer status of the patient. Omeprazole, esomeprazole, and rabeprazole demonstrate stronger competitive *CYP2C19* inhibition than lansoprazole and have significant impact in patients who are carriers of functional or increased function alleles (i.e., *CYP2C19* normal, rapid and ultrarapid metabolizers) compared to carriers of loss of function alleles (i.e., *CYP2C19* intermediate and poor metabolizers)^[55,63]. A prospective double-blind, crossover, randomized study, found that the use of esomeprazole significantly reduced platelet inhibition in clopidogrel-treated acute coronary syndrome patients who were carriers of *17 or *1/*1 compared with those treated with clopidogrel and placebo. This interaction was not observed among *2 carriers^[64].

CYP2C9

Clinically relevant inhibitors of *CYP2C9* include amiodarone, fluconazole, and miconazole. Co-administration of warfarin (*CYP2C9* substrate) and amiodarone (*CYP2C9* inhibitor) require adjustment of dose, which is accounted for in warfarin pharmacogenetic dosing algorithms^[65,66]. Likewise, fluconazole, an inhibitor of *CYP2C9* among other enzymes, produced a 2-fold increase in siponimod plasma concentrations among *CYP2C9* normal metabolizers. Notably, normal metabolizers of *CYP2C9* can be phenoconverted to intermediate or poor metabolizers^[67,68]. Commonly prescribed *CYP2C9* substrates like warfarin, phenytoin, proton pump inhibitors, and non-steroidal anti-inflammatory drugs should be monitored for phenoconversion^[62].

Thiopurine Methyltransferase

TPMT activity is important for substrates including mercaptopurine, azathioprine, thioguanine, and cisplatin^[69,70]. This enzyme's activity is affected by recent blood transfusions for which enzyme activity testing alone may result in inaccurate results unlike pharmacogenetic testing, which remains stable despite transfusion^[71]. However, co-administration with additional bone marrow suppressing drugs such as sulfasalazine, mesalamine, and olsalazine should be carefully monitored and incorporated into the interpretation of pharmacogenetic results^[54].

Moreover, social habits should also be taken into consideration. For instance, smoking induces *CYP1A2* which may be relevant for drugs affected by *CYP1A2* such as caffeine, clozapine, fluvoxamine, olanzapine, and theophylline^[72]. Phenoconversion is not permanent so once the interacting medication or habit is discontinued, the pharmacogenetic-guided therapeutic recommendation should be re-examined and corrected as necessary.

Organ transplant patients

In cases of stem cell, liver, and bowel transplant, special consideration should be taken when collecting and interpreting pharmacogenetic results. Clinicians must ensure that samples from stem cell transplant patients are collected prior to the transplant for DNA analysis. This guarantees that the transplant recipient's DNA is analyzed and not that of the transplant donor. Moreover, the liver and intestines possess *CYP3A* enzymes important for metabolism of many medications including tacrolimus. In addition, preliminary studies have found that formulation and route of tacrolimus influences the significance of *CYP3A5* pharmacogenetic results^[73,74]. When evaluating pharmacogenetic results for liver and bowel transplant recipients; clinicians should be aware that the genetic makeup of the new graft (i.e., donor's genotype) may be different from that of the transplant recipient. Hence, the use of only the recipient's genetic information may be misleading. Studies are underway to help unravel the pharmacogenetic complexities related to the type of transplant and the influence of the recipient versus the donor's genotypes as it relates to the timing of the transplant surgery and beyond.

Ontogeny

Neonatal and pediatric patients undergo age related development of metabolizing enzymes. For this reason, a general knowledge of the enzymes' maturation process is discussed here. A simplified categorization of the process of maturation can be grouped into three categories^[75]. The first are enzymes that are expressed at high levels during gestation and within 1-2 years after birth with *CYP3A7* being an example of this category. The second category comprises *CYP3A5* and *CYP2C19* which are expressed at constant levels during gestation. Unlike *CYP3A5*, *CYP2C19*'s expression increases throughout the first year of life. The third category are those enzymes that are not expressed or are at low levels during gestation and increase substantially during the first to second years of birth. Examples are *CYP3A4*, *CYP2C9*, *CYP2D6*, and *CYP2E1*^[62,75]. In general, ontogeny needs to be considered during evaluation of pharmacogenetic results for neonates, infants, and younger pediatric patients.

Pregnancy

Pregnancy results in a multitude of endocrine as well as metabolic changes that affect interpretation of pharmacogenetic results. Most *CYP450* enzymes are induced during pregnancy namely *CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2E1*, *CYP3A4*, and *CYP3A5*^[76]. *CYP2D6* enzyme levels are induced to higher levels in the second through third trimester for ultrarapid and normal metabolizers compared to first trimester^[77,78]. Clinically, this indicates that normal metabolizers of these enzymes would need monitoring for the duration of the pregnancy to ensure efficacy with active drugs such as antidepressants and antiemetics, and safety with prodrugs such as codeine, since pregnancy may induce *CYP2D6*, leading to a potential change in metabolizer status, affecting medications that are metabolized through *CYP2D6*^[57,79,60]. However, for pregnant women who are ultrarapid metabolizers, no further adjustments to the actionable pharmacogenetic recommendations would be anticipated. For instance, ondansetron would not be recommended for any *CYP2D6* ultra-rapid metabolizer regardless of pregnancy status^[77-79]. *CYP2D6* intermediate and poor metabolizers need no additional adjustments from guideline recommendations during pregnancy^[77,78]. Alternatively, inhibitory effects on *CYP1A2* and *CYP2C19* enzyme levels have also been reported during pregnancy^[77,80]. Use of caffeine (a substrate of *CYP1A2*) in the third trimester results in a 65% decreased metabolism as compared to non-pregnant women^[77]. The current limited evidence in pregnant women suggest that it is advisable to monitor normal metabolizers in addition to other actionable phenotypes to ensure that all clinically-relevant factors are carefully considered when utilizing pharmacogenetic results to make therapeutic decisions in pregnancy.

CONCLUSION

In summary, pharmacogenetic testing is a valuable tool that should be adopted into routine clinical practice. We provide a review of the preparatory steps necessary to implement a clinical pharmacogenetic

program and other pertinent clinical considerations to maximize the impact of pharmacogenetic testing in clinical practice [Table 1]. We comment on choosing the gene(s) and variants to test, the patient population, available resources, and the impact of phenoconversion, organ transplant, and pregnancy on pharmacogenetic applications. At its core, it is about the individual and how best we can optimize therapeutic recommendations for each patient. Yet, the achievement of this goal is largely driven by a team effort in which pharmacists play vital roles. Pharmacogenetics is not the ultimate decision maker when it comes to making prescribing decisions. Other intrinsic and extrinsic factors should also be considered along with the pharmacogenetic information to make an informed decision on the optimal therapeutic regimen for each patient.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the paper: Owusu Obeng A, El Rouby N, Liu M, and Wallsten R

Provided administrative support: Owusu Obeng A, El Rouby N

Provided technical support: Owusu Obeng A, El Rouby N, Liu M, Wallsten R

Contributed to the writing, review and final approval of the manuscript: Owusu Obeng A, El Rouby N, Liu M, Wallsten R

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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