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ACMG TECHNICAL STANDARD

Clinical pharmacogenomic testing and reporting: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

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Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with this technical standard. They also are advised to take notice of the date any particular technical standard was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

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ABSTRACT

Pharmacogenomic testing interrogates germline sequence variants implicated in interindividual drug response variability to infer a drug response phenotype and to guide medication management for certain drugs. Specifically, discrete aspects of pharmacokinetics, such as drug metabolism, and pharmacodynamics, as well as drug sensitivity, can be predicted by genes that code for proteins involved in these pathways. Pharmacogenomics is unique and differs from inherited disease genetics because the drug response phenotype can be drug-dependent and is often unrecognized until an unexpected drug reaction occurs or a patient fails to respond to a medication. Genes and variants with sufficiently high levels of evidence and consensus may be included in a clinical pharmacogenomic test; however, result interpretation and phenotype prediction can be challenging for some genes and medications. This document provides a resource for laboratories to develop and implement clinical pharmacogenomic testing by summarizing publicly available resources and detailing best practices for pharmacogenomic nomenclature, testing, result interpretation, and reporting.

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Background

Purpose of pharmacogenomic testing

The goal of clinical pharmacogenomic testing is to examine genetic variants that are associated with interindividual variability in discrete aspects of pharmacology such as drug metabolism.[1,2] Pharmacogenomic variants can explain some drug response phenotypes, including therapeutic failure, side effects and/or adverse events, or nonstandard dosing. The commonly tested genes are often involved in pharmacokinetic (drug inactivation or activation) or pharmacodynamic (drug response) pathways. Pharmacokinetic processes include drug absorption, distribution, metabolism, and elimination, whereas pharmacodynamic processes explain the mechanism(s) of action that define therapeutic response and adverse events. Common examples of pharmacogenomic biomarkers include enzymes, receptors, ion channels, transporter proteins, and immune mediators. Genes of pharmacogenomic relevance are often referred to as pharmacogenes.[3,4]

Correlating a pharmacogenomic test result with a drug response phenotype can pre-emptively guide drug selection and/or dosing decisions for certain commonly prescribed medications that have a US Food and Drug Administration (FDA) drug label or other professional society therapeutic recommendations. As such, the identification of certain pharmacogenomic variants may indicate that a patient should avoid being prescribed a specific medication on the basis of efficacy concerns or risk of experiencing serious side effects or adverse events. In addition, the identification of certain pharmacogenomic variants could suggest that a patient may benefit from nonstandard doses of a medication. Using pharmacogenomic information can therefore contribute to what is often referred to as personalized or precision medicine or individualized drug therapy.[3,4]

There are many publicly available resources that support clinical pharmacogenomic testing (Box 1). For example, the Pharmacogenomic Knowledgebase (PharmGKB) is an international, publicly accessible repository that aggregates, integrates, and disseminates descriptions and references for gene–drug associations; catalogs gene-based dosing guidelines; and lists approved drug labeling that contain pharmacogenomic information.[5] The Clinical Pharmacogenetics Implementation Consortium (CPIC) creates evidence-based gene/drug clinical practice guidelines.[6-8] Notably, CPIC does not make recommendations as to whether pharmacogenomic testing should be performed or which variants should be tested. Rather, CPIC provides recommendations on how to translate genetic test results into actionable prescribing decisions for specific drugs to enable the treating clinical professional to optimize drug therapy. Another pharmacogenomic resource is the Pharmacogene Variation (PharmVar) Consortium that serves as a centralized data repository cataloging high-quality variation data of pharmacogenes, providing a unifying allele designation system (or nomenclature) for a growing number of genes.[9-14]

In addition to PharmGKB, CPIC, and PharmVar, the FDA has published resource tables for pharmacogenomic biomarkers. The FDA "Table of Pharmacogenomic Biomarkers in Drug Labeling" lists both somatic and germline biomarkers on the basis of information contained within the approved drug labeling.[15] In addition, the FDA "Table of Pharmacogenetic Associations" provides 3 germline gene–drug association tables based on clinical evidence: (1) gene–drug associations with therapeutic management recommendations, (2) gene–drug associations with potential

Box 1. Pharmacogenomic resources

- Pharmacogenomics Knowledgebase (PharmGKB)
  https://www.pharmgkb.org/
- Clinical Pharmacogenetics Implementation Consortium (CPIC)
  https://cpicpgx.org/
- Pharmacogene Variation Consortium (PharmVar)
  https://www.pharmvar.org/
- US Food and Drug Administration (FDA) Table of Pharmacogenomic Biomarkers in Drug Labeling
  https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling
- FDA Table of Pharmacogenetic Associations
  https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations
- The Genetic Testing Reference Materials Coordination Program (GeT-RM)
  https://www.cdc.gov/labquality/get-rm/inherited-genetic-diseases-pharmacogenetics/pharmacogenetics.html
- Drug Interactions Flockhart Table
  https://drug-interactions.medicine.iu.edu/MainTable.aspx
- Association for Molecular Pathology Clinical Practice Committee's Pharmacogenomics (PGx) Working Group
  https://www.amp.org/clinical-practice/practice-guidelines/
- Dutch Pharmacogenetics Working Group
  https://www.pharmgkb.org/page/dpwg

impact on safety or response, and (3) gene–drug associations with pharmacokinetic relevance only.[16] Of note, there is substantial but not complete overlap between the gene–drug associations included in the FDA tables and CPIC guidelines because their evaluations of evidence and criteria differ, as well as the recommendations from other professional societies.[17]

This report provides an update to the 2012 American College of Medical Genetics and Genomics (ACMG) guideline on *CYP2D6* testing for tamoxifen therapy[18] and expands the scope to provide guidance and professional recommendations on implementing pharmacogenomic testing among clinical laboratories with a focus on current best practices, including standardized pharmacogene nomenclature, testing methodologies and validation, and data interpretation and reporting.

Pharmacogenomic nomenclature

Nomenclature for certain pharmacogenes is focused on defining haplotypes that are made of 1 or more single nucleotide variants (SNVs) or small insertions/deletions, typically less than 50 nucleotides. In a commonly used pharmacogenomic nomenclature system, combinations of sequence variants (ie, haplotypes) are often designated by star (*) alleles. The **1* allele is assigned as a default if none of the tested variants are detected (an exception being *NAT2* for which the default assignment is the **4* allele). The PharmVar Consortium is systematically cataloging star alleles for the *CYP* genes[10,11] but has expanded its database to other important pharmacogenes such as *NUDT15*[19] and *SLCO1B1*.[10] Although numerous variants may be present on a given star allele, those that cause an amino acid change or frame-shift or are known to impact splicing or expression levels are designated as core variants. These core variants are typically the analytical target for clinical pharmacogenomic testing assays. A star allele may have 2 or more suballeles.[12] For instance, *CYP2D6*4* is defined by the c.506-1G>A splice variant (rs3892097, NC_000022.11:g.42128945C>T) that causes loss of function. This variant has a relatively high frequency in the general population (eg, approximately 20% in non-Finnish Europeans and approximately 8% in African Americans; https://gnomad.broadinstitute.org/variant/22-42128945-C-T?dataset=gnomad_r3) and has been found in numerous haplotypes in combination with additional SNVs. These haplotypes are referred to as *CYP2D6*4* suballeles. The core allele is defined as *CYP2D6*4*, and suballeles are differentiated by a numeric extension (eg, *CYP2D6*4.001*, **4.002*, etc.). All suballeles listed under a star number are assumed to be functionally equivalent. It is noteworthy that another common variant, c.100C>T (p.Pro34Ser, rs1065852, NC_000022.11:g.42130692G>A), which is classified as a core variant of the *CYP2D6*10* allele among several others, is part of all but one *CYP2D6*4* suballele, ie, *CYP2D6*4.012* (formerly *CYP2D6*4M*, https://www.pharmvar.org/gene/CYP2D6). As such, detecting both the c.506-1G>A and c.100C>T variants as heterozygous, suggests that the patient's diplotype is either *CYP2D6*4/*10* or *CYP2D6*1/*4*, depending on whether the variants are on the same chromosome (in *cis*) or opposite chromosomes (in *trans*). Most *CYP2D6* testing does not include phasing to determine if they are in *cis* or in *trans*; however, allele frequency data suggest that these 2 variants are most often in *cis*. Consequently, ACMG recommends reporting *CYP2D6*1/*4* rather than *CYP2D6*4/*10* when the 2 variants are detected as heterozygous.[10,12,13] And generally, when phasing is ambiguous, population data should be used to report the most likely genotype, and disclaimers should be included in reporting related to phasing assumptions. It is also important to note that some sites of variation may have more than 1 allele in the population (eg, A>G/C/T), and thus, the database of single nucleotide polymorphisms (dbSNP) rs identifier, which is a genomic location identifier, is not necessarily a unique representation of variation and may be ambiguous regarding the variant that is present or tested. As such, the use of dbSNP rs identifiers should not be the only representation of the variant, and more specific nomenclature must be provided.

In addition, some pharmacogenes are affected by copy number variants (CNVs), including gene deletions, duplications, multiplications, and gene rearrangements. For *CYP2D6*, a comprehensive summary of these CNVs is provided by the "Structural Variation" document on the PharmVar *CYP2D6* gene page (https://www.pharmvar.org/gene/CYP2D6). Although currently there is no standardized method to annotate *CYP2D6* structural variants, CNVs are commonly indicated by placing a multiplication sign and the number of gene copies after the affected allele. For example, *CYP2D6*1/*2×2* indicates that there are 2 copies of the **2* allele on the same chromosome for 3 total gene copies, whereas *CYP2D6*1/*36+*10* indicates that 1 chromosome has a copy of the *CYP2D6*36* allele in tandem with a *CYP2D6*10* allele for a total of 3 gene copies. The PharmVar GeneFocus review series provides additional information on the nomenclature of pharmacogenes, which currently have been reported for *CYP2D6*,[13] *CYP2C19*,[9] *CYP2B6*,[11-13] and *CYP2C9*.[14]

Importantly, given that **1* is usually the default allele assignment for genotyping assays (ie, none of the tested variants were detected), there is a residual risk that the individual has a rare non-**1* genotype depending on how comprehensive the test is. Residual risk for having a non-**1* allele, despite testing negative for the variants interrogated, is dependent on the number of alleles tested and allele frequencies. For example, the defining variants for the normal function *CYP2D6*2* allele are p.Arg296Cys (rs16947, NC_000022.11:g.42127941G>A)[13] and p.Ser486Thr (rs1135840, NC_000022.11:g.42126611C>G).[13] However, these 2 variants also occur on other alleles such as *CYP2D6*8*, **11*, **12*, **17*, **29*, and many others, in addition to the unique core SNVs on each allele. Failure to capture these additional core SNVs during testing may result in a *CYP2D6*2* default assignment and misclassification of a nonfunctional allele as a normal function allele and thus

produce a genotype call that may lead to an inaccurate phenotype prediction. Therefore, it is important for clinical laboratories to consider such issues when selecting and/or developing a pharmacogenomic testing platform.

Pharmacogenomic Testing Methodologies

Pharmacogenomic testing

The analytical strategy used in pharmacogenomic testing depends on a variety of factors, such as the complexity of the gene; the extent, frequency, and type of genetic variation; and the time needed for return of the results.

Targeted pharmacogenomic testing

Targeted genotyping assays typically only test for selected star allele haplotypes cataloged by PharmVar. If the need for a rapid test result is clinically indicated, an assay may be designed to limit complexity, such as through targeted detection of a small number of the most common and clinically relevant variants. Commercially available in vitro diagnostic products are available for some pharmacogenomic applications and may reduce complexity of testing and data analysis to reduce the time to result.[20] However, many pharmacogenomic assays are based on laboratory-developed test approaches where assays are developed, validated, and performed by clinical testing laboratories. Clinical laboratories that offer pharmacogenomic testing can be found through the voluntary National Institutes of Health Genetic Testing Registry.[21]

Most clinical pharmacogenomic testing involves the analysis of specific targeted genes and variants. Orderable tests may include a single gene or gene panels where specific variants are interrogated. Targeted genotyping will not detect any variant or allele that is not directly tested, and therefore, a negative genotyping result does not rule out the possibility that an individual harbors another variant not interrogated by the assay. For example, reporting a **1* result by a genotyping platform indicates that none of the targeted variants were detected, and it does not necessarily mean that there are no variants present in the gene.

ACMG recommendations for targeted pharmacogenomic testing, consistent with the consensus guidelines provided by the Association for Molecular Pathology (AMP) and the College of American Pathologists (CAP),[22-24] include the following:

- Select clinically relevant pharmacogenes and document the evidence and rationale supporting the gene and variant selection.
- Include clinically relevant variants based on allele function, allele frequencies in all populations, and availability of reference materials.
- List all interrogated variants that can be detected by the targeted assay in the test description.

Exome and genome sequencing

Exome sequencing (ES)–based tests identify variants within the coding regions of genes and variants at exon/intron junctions that may impact messenger RNA splicing. This approach does not identify deep intronic variants or those situated further upstream unless the assay was specifically modified to include specific non-coding variants. However, such variants are readily detected by genome sequencing (GS) or targeted next-generation sequencing–based gene panels that have been designed to cover SNVs located outside coding regions. Structural variants in drug metabolizing enzymes can be detected by GS using bioinformatic tools[25-28] but are most often detected using commercially available gene-specific copy number assays (eg, quantitative PCR, targeted arrays).[29]

High throughput sequencing platforms that use target enrichment have limitations, including decreased coverage in regions with high GC content (eg, the 5′ end of genes), limited detection of CNVs, reduced sensitivity to detect insertions/deletions, and interference from homologous pseudogenes. These technical limitations are expected to improve over time, if not resolve; however, rare and novel variants will likely be of uncertain clinical significance and challenging to interpret. Potentially novel haplotypes may also be identified by ES, GS, or panel-based analyses; however, additional characterization may be necessary to submit a novel allele to PharmVar.[11,12] Of note, most laboratories currently perform targeted pharmacogenomic testing; however, this is an evolving area, and as more laboratories employ full-gene sequencing and exome or genome testing, reporting novel/rare variants of uncertain clinical significance may be preferred. Nevertheless, given the current lack of professional standards and guidelines for the clinical classification of pharmacogenomic variants,[30] laboratories should approach this cautiously and appropriately document their policies and classification criteria.

ACMG recommendations for pharmacogenomic ES and GS include the following:

- Report clinically relevant pharmacogenomic variants (and inferred haplotype/diplotypes when possible) and document the evidence and rationale supporting the gene and variant selection.
- Specify types of variants (SNVs and CNVs) that can be detected by the assay.
- List the reportable pharmacogenomic variants and limitations of these assays.

Pharmacogene copy number variation

Germline structural variants can range in size from kilobases to several megabases, which include deletions, duplications, insertions, inversions, and other complex rearrangements.

CNVs have been reported for some pharmacogenes with varying population frequencies. The most notable pharmacogene affected by structural variation is *CYP2D6*.[13,31-33] Specifically, the *CYP2D* locus on chromosome 22 harbors 2 highly homologous pseudogenes, *CYP2D7* and *CYP2D8*, which are closely located and evolutionarily related to the functional *CYP2D6* gene.[13] These pseudogenes enable meiotic nonallelic homologous recombination that can result in germline *CYP2D6* deletions, duplications, and multiplications and *CYP2D6/CYP2D7* gene conversions. Summaries of *CYP2B6*, *CYP2C19*, and *CYP2D6* CNVs can be found in the "Structural Variation" documents available on their respective PharmVar gene pages and PharmVar GeneFocus reviews.[9,13,34]

ACMG recommendations for pharmacogenomic CNV testing and reporting include the following:

- Report CNVs of clinically relevant pharmacogenes (and their star (*) allele haplotypes) as appropriate.
- Determine the minimum size of the CNV that can be detected by the assay when possible.
- List all reportable CNVs that can be detected by the assay in the test description.
- Laboratories must consider the complexities, capabilities and limitations of the assay, and the current state of knowledge.

Validation of a Clinical Pharmacogenomic Test

Performance characteristics

Laboratories should establish the performance characteristics of their pharmacogenomic platform during validation. Performance characteristics include the accuracy and precision of results, the analytical sensitivity and specificity, and the reportable ranges. The accuracy evaluation is accomplished by running a minimum of 20 previously characterized samples (CAP checklist COM.40350 revised on June 24, 2020) that represent alleles/haplotypes that can be detected by the platform, including SNVs and CNVs. However, a pharmacogenomic assay validation study with more samples is preferred to demonstrate analytical accuracy of the test method, particularly for structural variants. The reportable range of results should include criteria to identify a reportable allele/haplotype. The laboratory must document the concordance of the expected results and any unexpected findings. Quality control and quality assurance metrics vary on the basis of the method(s) employed by the laboratory and the relevant regulatory oversight, which are beyond the scope of this document. In addition, ongoing participation in a proficiency testing program (eg, CAP) is standard practice and ensures performance characteristics and quality assurance.

Specimen types

Different specimen types may be used with the pharmacogenomic platform employed by a laboratory. However, it is expected that the initial test validation will involve the most common specimen type for the expected intended use (eg, DNA extracted from peripheral blood). For alternative DNA sources, the laboratory should determine the performance characteristics for each specimen type. If there are minimal or no changes to the processing or analysis, then running a minimum of 3 known samples is recommended to validate a new DNA source; however, additional samples may be required for thorough assessment of CNVs. If significant changes are made in the processing or analysis procedures, then a new validation for the new DNA source, in accordance with CAP requirements, is required.

Reference materials

The Genetic Testing Reference Materials Coordination Program is a combined effort among the Centers for Disease Control and Prevention-based Genetic Testing Reference Materials Coordination Program, Coriell Institute for Medical Research, and members of the pharmacogenomic community.[35] Considering the growing use of pharmacogenomic testing, established sets of well-characterized reference materials are needed for assay development, validation, quality control, and proficiency testing. To address the increasing need for reference materials, genomic DNA samples were characterized for pharmacogenes and consensus genotypes established.[36,37] Although the most common variants were assayed in the first 2 projects, many rare alleles were not identified among the samples tested, and a third project characterized several rare and complex alleles to complement the existing materials for *CYP2D6*.[29] Clinical and research laboratories can acquire these publicly available materials from the Coriell Institute (Camden, NJ).

Pharmacogenomic Reporting and Result Interpretation

Pharmacogenomic reporting

Given the challenges surrounding the reporting of pharmacogenomic test results, significant effort has been made to create standardized guidelines. A pharmacogenomic workgroup led by the Centers for Disease Control and Prevention recommended that pharmacogenomic test reports should adopt standardized test results for the purpose of transparency and accessibility to geneticists. Essentially, these recommendations include the use of gene names per the Human Genome Organisation Gene Nomenclature Committee as well as the variant's dbSNP rs identifier, report

sequence variants using Human Genome Variation Society nomenclature, specify the reference sequence used to call variants, indicate each variant and/or haplotype observed in the test report, list variants and haplotypes that can be detected by the test, describe test limitations, and have the test description publicly available.[38] Additional expert workgroup recommendations have been made to standardize pharmacogenomic phenotype descriptors.[4,39,40] These ongoing efforts to standardize how pharmacogenomic variants are described and reported allows for more consistent results and the continued adoption and growth of pharmacogenomic testing.[41]

ACMG recommendations for pharmacogenomic reporting include the following:

- Utilize the HUGO Gene Nomenclature for gene names.
- Report sequence variants using HGVS nomenclature (reporting dbSNP rs identifier is optional).
- Specify the reference sequence used to call variants.
- List each variant and/or haplotype observed in the test report.
- Describe test limitations in the report.
- List all variants and haplotypes that can be detected by the test.
- Provide a test description that is publicly available.

Phenotype prediction from pharmacogenomic test results

Pharmacogenomic test results (ie, genotypes) are typically used to infer a patient phenotype or metabolizer status. There are 5 CPIC-recommended phenotype groups for specific drug metabolism genes,[39] including poor metabolizer, intermediate metabolizer, normal metabolizer (NM), rapid metabolizer, and ultrarapid metabolizer (UM). Phenotype prediction is dependent on the identified diplotype: for example, 2 nonfunctional alleles result in a poor metabolizer phenotype, whereas 2 normal function or 1 normal function and 1 decreased function alleles result in an NM phenotype. Patients with an intermediate metabolizer phenotype may have 1 normal function allele in combination with 1 nonfunctional allele or have 2 decreased function alleles. In addition, 3 copies of a normal function allele, regardless of the other allele, result in a UM phenotype.[42] For example, *CYP2D6*1/*2×2* indicates the presence of a total of 3 gene copies, and because the *CYP2D6*2* allele has normal function, this duplication in combination with a *CYP2D6*1* allele translates into a UM phenotype. Another example of a CNV-containing genotype is *CYP2D6*1/*36+*10*. Although this genotype also features a total of 3 gene copies, subjects with this genotype are NMs because the **36* allele is a nonfunctional allele and the **10* allele is a decreased function allele. Of note, genotype to phenotype translation tables are available from PharmGKB and CPIC (Box 1). To facilitate the translation of genotype to phenotype for certain pharmacogenes (eg, *CYP2D6* and *CYP2C9*), the activity score (AS) system is used by CPIC for phenotype prediction. Briefly, each allele is assigned an activity value, and the sum of the activity values is the AS for the specific genotype (https://www.pharmgkb.org/page/pgxGeneRef).[40,43] Importantly, CPIC has published consensus recommendations for the translation of genotype to phenotype using the AS system for several genes,[40] which should enable more consistent reporting across clinical laboratories. The ACMG encourages the use of the CPIC AS system for genotype to phenotype translation of relevant pharmacogenes (eg, *CYP2D6, CYP2C9, DPYD*).

Clinical interpretation of pharmacogenomic variants

The clinical interpretation and reporting of pharmacogenomic test results is an evolving area that currently does not have formal or standardized best practices. In addition, the FDA issued a warning statement in 2018 related to pharmacogenomic testing, specifically highlighting concerns over inappropriate claims in some genetic test reports that predict patient drug responses (https://www.fda.gov/news-events/press-announcements/fda-issues-warning-letter-genomics-lab-illegally-marketing-genetic-test-claims-predict-patients). However, AMP issued a position statement in 2019 detailing their best practices in pharmacogenomic testing (https://www.amp.org/AMP/assets/File/position-statements/2019/Best_Practices_for_PGx_9_4_2019.pdf?pass=96), which also included a recommendation on pharmacogenomic test reporting. AMP recommends reporting genotype and metabolizer phenotypes (where applicable), a list of medications that may be affected by the identified genotype, a generalized statement if an alternative therapy may be considered on the basis of the results, and a list of resources that could inform actionable decisions (eg, CPIC guidelines).

Given that the FDA has recently issued explicit pharmacogenomic tables that are stratified by level of evidence, the ACMG recommends inclusion of pharmacogenomic result interpretation and reporting, consistent with the AMP recommendations. In addition, the FDA therapeutic management recommendations that currently have supportive evidence are recommended to be included in clinical pharmacogenomic test reports. Therapeutic recommendations that the FDA has stated have "potential impact on safety or response," and those listed with a "potential impact on pharmacokinetic properties" are not currently recommended to be explicitly stated in pharmacogenomic test reports issued by genetic testing laboratories.[16] In addition to these recommendations, consistent with the AMP reporting recommendations, the ACMG also encourages listing additional recommendation resources, particularly high evidence drug–gene pairs (eg, FDA, CPIC).

It is important to note that the accuracy of phenotypic prediction is dependent on the variants detected as well as on the drug substrate in question. In addition, drug–drug interactions can dramatically alter the metabolizer

Box 2. Tamoxifen and *CYP2D6*

- US Food and Drug Administration listed tamoxifen as a drug for which the data demonstrate a potential impact on pharmacokinetic properties only.[16]
- Tamoxifen is metabolized to more potent antiestrogenic metabolites by CYP2D6; star alleles conferring decreased enzyme activity are associated with reduced tamoxifen efficacy.
- To date, over 140 *CYP2D6* alleles have been designated by Pharmacogene Variation (PharmVar),[11] including many no function (eg, *CYP2D6*4*), decreased function (eg, *CYP2D6*10*), and increased function alleles (eg, *CYP2D6*2x2*); genotype to phenotype translation and allele functionality tables are available through Pharmacogenomics Knowledgebase.[5]
- Some drugs are CYP2D6 inhibitors and can cause phenocopy, ie, convert a ultrarapid metabolizer, normal metabolizer, or intermediate metabolizer into a poor metabolizer.
- Recommendations based on the 2018 Clinical Pharmacogenetics Implementation Consortium guideline for women with surgically resected ER+/HER2– breast cancer: consider use of an alternative hormonal therapy (aromatase inhibitor) or a higher tamoxifen dose (40 mg/day) for patients with *CYP2D6* genotypes associated with decreased CYP2D6 metabolism. Potent CYP2D6 inhibitors should be avoided.[44]

phenotype. For example, a patient on tamoxifen therapy who is also prescribed a strong CYP2D6 inhibitor (eg, paroxetine) may be at increased risk for tamoxifen treatment failure because of an inability to produce adequate levels of endoxifen (Box 2). In this example, the patient's genotype would not be the only predictor of phenotype. Therefore, laboratories should not include suggestions for specific patient-specific dosages. Importantly, clinical pharmacogenomic test reports should uniformly state that drug response phenotypes can be influenced by multiple clinical factors and that any medication management inferred from the pharmacogenomic test result should take these variables into consideration and be at the discretion of the managing clinical professional.

ACMG recommendations for pharmacogenomic result interpretation and reporting include the following:

- Report genotype and metabolizer phenotypes (where applicable).
- Provide a list of medications that may be affected by the identified genotype.
- Provide a generalized statement if an alternative therapy may be considered based on results.
- Provide a list of resources that could inform actionable decisions (eg, FDA tables, CPIC guidelines).

Box 3. Thiopurines (*TPMT* and *NUDT15*)

- US Food and Drug Administration approved labeling and Clinical Pharmacogenetics Implementation Consortium recommend dose adjustment of thiopurines (azathioprine, mercaptopurine, thioguanine) for patients with cancer with reduced TPMT or NUDT15 activity (poor metabolizer or intermediate metabolizer) or using alternative nonthiopurine immunosuppressants for nonmalignant conditions.[16,46]
- For patients prescribed thiopurines, carrying no function and/or decreased function *TPMT* and the *NUDT15* alleles, have a higher risk of experiencing myelosuppression.
- *TPMT*2*, *TPMT*3A*, and *TPMT*3C* alleles account for about 95% of individuals with reduced levels of TPMT activity.
- The *NUDT15*3* allele is the most commonly observed nonfunctional *NUDT15* allele.

- Clinical pharmacogenomic test reports should not provide patient-specific dosing.
- Include FDA therapeutic management recommendations in the clinical pharmacogenomic test report that currently have supportive evidence.
- Clearly state in the clinical pharmacogenomic test report that the accuracy of phenotypic prediction is dependent on the variants detected, as well as on the drug substrate if applicable.
- Clearly state in the clinical pharmacogenomic test report that drug–drug interactions can alter the metabolizer phenotype.

Examples of clinical pharmacogenomic guidelines

There are several examples of pharmacogenes where clinical guidelines have been established on the basis of

Box 4. Siponimod and *CYP2C9*

- US Food and Drug Administration approved labeling for siponimod (Mayzent) recommends maintenance dosing for patients on the basis of *CYP2C9* genotypes: 2 mg/day for *CYP2C9*1/*1*, *1/*2*, and *2/*2*; 1 mg/day for *CYP2C9*1/*3* or *2/*3*; and contraindicated for *CYP2C9*3/*3*.[47]
- Several *CYP2C9* alleles included in tier 1 testing recommendations[23] that infer no function (*CYP2C9*6*) and decreased function (*CYP2C9*5*, *8*, *11*) are not included in the current drug labeling; these alleles are relatively common in people of African ancestry, including African Americans.[23,48]
- Drug–drug interactions are also important considerations for dose optimization.[47,49]

Box 5. Carbamazepine (*HLA-B*, *HLA-A*)

- US Food and Drug Administration approved labeling recommends to avoid using carbamazepine on the basis of the *HLA-B*15:02* and *HLA-A*31:01* genotypes, where data support or indicate a potential effect on drug safety.[16]
- *HLA-B*15:02* genotype increases risk for Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), and the *HLA-A*31:01* genotype increases risk for SJS, TEN, drug reaction with eosinophilia and systemic symptoms, and maculopapular exanthema.
- Recommendations based on the 2017 Clinical Pharmacogenetics Implementation Consortium guideline[50]: If *HLA-B*15:02* and/or *HLA-A*31:01* genotypes are present, do not use carbamazepine for naive patients or cautiously consider using it if previously used consistently for >3 months. If alternative agents are not available and patient is positive for *HLA-A*31:01*, consider using carbamazepine with increased clinical monitoring.

either prospective clinical trial data (level 1 data) or secondary analysis of prospective clinical trials (level 2).[45] Some of the most well-known examples are in the setting of oncology (Boxes 2 and 3), neurology (Boxes 4 and 5), and cardiology (Box 6), where pre-emptive or reactive testing in the setting of drugs with narrow therapeutic indices can prevent severe side effects, adverse events, and/or treatment failures.

Box 6. Clopidogrel and *CYP2C19*

- US Food and Drug Administration approved labeling recommends to avoid using clopidogrel (Plavix) in patients with 2 no function alleles of the *CYP2C19* gene.
- *CYP2C19* poor metabolizers (**2/*2*, **2/*3*, **3/*3*) exhibit diminished platelet inhibition, enhanced platelet aggregation, and higher rates of coronary events when treated with clopidogrel after percutaneous intervention.[51]
- A meta-analysis of 7 randomized controlled trials (15,949 patients) showed that ticagrelor and prasugrel compared with clopidogrel significantly reduced ischemic events (relative risk = 0.70; 95% CI = 0.59-0.83) in individuals who have *CYP2C19* loss-of-function variants but not in individuals where no such variants have been identified (relative risk = 1.0; 95% CI = 0.80-1.25).[52]

Conclusion

Characterization of pharmacogenomic diversity is challenging, when taking into account the numerous pharmacogenomic alleles and suballeles that have been reported across populations.[5,11,37,53] Laboratories should understand the challenges involved in pharmacogenomic testing and be familiar with allele nomenclature, technical limitations of genotyping and sequencing platforms, and issues related to result interpretation and reporting. A variety of analytical platforms are available for pharmacogenomic testing, each with its own technical limitations. The issue of not detecting clinically relevant rare variants may be resolved as laboratories evolve from targeted genotyping[54] to full gene sequencing or gene panels that allow for more comprehensive SNV and CNV calling.[55-58] These techniques, however, impose new challenges such as interpreting rare variants with unknown function and/or phenotypic effect.[55,59] Guidance for application of multiple gene variants to a single drug response phenotype is also largely undefined. Taken together, these challenges should be considered by clinical laboratories to determine what pharmacogenomic methodology is most appropriate to be validated as a clinical pharmacogenomic test.

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Conflict of Interest

M.K.T., E.L., G.A.M., S.R., V.M.P., and S.A.S. serve as directors in clinical laboratories that perform a breadth of genetic and genomic analyses on a fee for service basis. The other authors declare no conflicts of interest.

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