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Pharmacogenomic Testing in the Clinical Laboratory: Historical Progress and Future Opportunities

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Pharmacogenomics is a rapidly evolving field with a strong foundation in basic science dating back to 1960. Pharmacogenomic findings have been translated into clinical care through collaborative efforts of clinical practitioners, pharmacists, clinical laboratories, and research groups. The methods used have transitioned from targeted genotyping of relatively few variants in individual genes to multiplexed multi-gene panels, and sequencing-based methods are likely on the horizon; however, no system exists for classifying and reporting rare variants identified via sequencing-based approaches. Laboratory testing in pharmacogenomics is complex for several genes, including cytochrome P450 2D6 (*CYP2D6*), *HLA-A*, and *HLA-B*, owing to a high degree of polymorphisms, homology with other genes, and copy-number variation. These loci require specialized methods and familiarity with each gene, which may persist during the transition to next-generation sequencing. Increasing implementation across laboratories and clinical facilities has required cooperative efforts to develop standard testing targets, nomenclature, and reporting practices and guidelines for applying the results clinically. Beyond standardization, harmonization between pharmacogenomics and the broader field of genomic medicine may be essential for facilitating further adoption and realizing the full potential of personalized medicine. In this review, we describe the evolution of clinical laboratory testing for pharmacogenomics, including standardization efforts and the anticipated transition from targeted genotyping to sequencing-based pharmacogenomics. We speculate on potential upcoming developments, including pharmacoepigenetics, improved understanding of the impact of non-coding variants, use of large-scale functional genomics to characterize rare variants, and a renewed interest in polygenic risk or combinatorial approaches, which will drive the progression of the field.

Key Words: Genotyping, PGx, Pharmacogenetics, Pharmacogenomics, Precision medicine, Standardization

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INTRODUCTION

Pharmacogenomics is a dynamic field that started with a strong research foundation and has been translated into the clinic, with many pharmacogenomic tests available today to facilitate treatment selection in clinical care. The field continues to grow and evolve through regular interactions between clinical practitioners, pharmacists, clinical laboratories, and research groups.

The term "pharmacogenetics" was first coined by Friedrich Vogel in 1959 to describe a new type of research aimed at better understanding the effects of genetic variation on the response to medications [1]. An article published in 1961 by Evans and Clarke in the *British Medical Bulletin* is the earliest publication on pharmacogenetics in PubMed [2]. Since the publication of

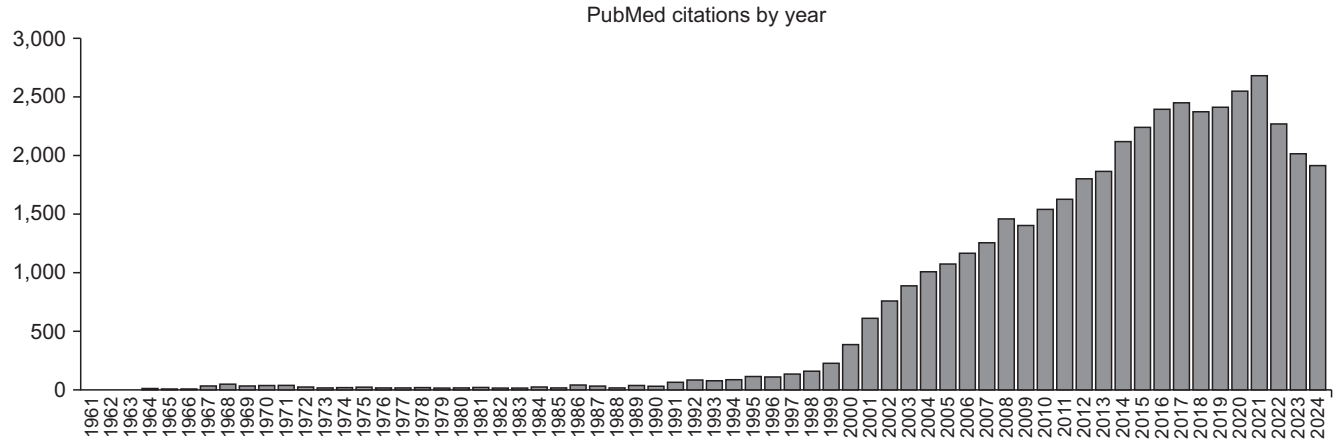


Fig. 1. Number of citations in PubMed returned from a search of "pharmacogenetics or pharmacogenomics" by year is displayed, from the first citation in 1961 up to 11/27/2024.

these early articles that launched the field, a steady but relatively small number of manuscripts was published each year on this topic until the early 2000s when the field advanced rapidly with a considerable increase in the number of publications (Fig. 1). This acceleration was partly attributed to the Human Genome Project in the United States, which drove awareness of all facets of genetics and perhaps led to the introduction of the term "pharmacogenomics" to capture the growing interest in evaluating broader genetic contribution to drug response beyond the metabolic genes recognized at the time. In addition, in the late 1990s and early 2000s, few laboratories began to offer pharmacogenetic tests (Fig. 2). Since the early implementation of pharmacogenetic testing, the methods have changed and enabled more complex testing involving multiple genes.

In this review, we describe the evolution of clinical laboratory pharmacogenomic testing (Fig. 2), including standardization efforts and the early stages of the transition from targeted-genotyping technology to sequencing-based pharmacogenomics. We also speculate on potential upcoming developments that will advance the field.



Fig. 2. Timeline of advancements in pharmacogenomics.

EARLY CLINICAL IMPLEMENTATION OF PHARMACOGENETICS

By the mid-to-late 1990s, pharmacogenetic research had advanced sufficiently to justify the implementation of clinical testing [3, 4] (Fig. 2). The Food and Drug Administration (FDA) and drug manufacturers recognized the impact of genetic variation on the safety and efficacy of some medications; however, the specific information required and the stage at which it should be collected and presented during the drug approval process remained uncertain [5]. The industry was concerned that a requirement for genetic data at the end of a clinical trial could hinder the approval of new medications when the DNA samples had not been stored. Additionally, new requirements for genetic

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analysis might lengthen the drug approval process [5]. Although pharmacogenomics may help identify population subsets for whom a certain medication is unsuitable, potentially signaling the decline of the "blockbuster" drug model, pharmaceutical companies have cautiously adopted a more optimistic stance. The ability to use genetic testing to identify individuals at risk for toxicity has been recognized as a strategy to facilitate the approval of medications that might otherwise fail to meet acceptable safety standards.

For existing medications, the early clinical implementation of pharmacogenomics to guide therapy for individual patients was limited to a small number of academic laboratories. One of the first examples of clinical pharmacogenetic testing was red blood cell phenotyping to determine thiopurine methyltransferase (TPMT) activity and adjust thiopurine dosing [6, 7]. Although TPMT-activity measurements technically involve biochemical assays, researchers recognized a trimodal level of red cell TPMT activity and hypothesized that a single gene with autosomal codominant inheritance may underlie the observed individual differences. Ultimately, variants in *TPMT* that segregated with this phenotype were identified, leading to the development of tests for genetic variants rather than enzyme activity [8].

Laboratories implementing pharmacogenomics developed tests independently that were generally limited to a single gene for a specific drug. This independent approach led to variability in terms of which alleles were tested [9] and how the results were reported [10]. Variants shown to impact drug metabolism through research (and thus clinically implemented) were typically relatively common variants or single-nucleotide polymorphisms (SNPs). Although haplotype structures were beginning to become recognized, owing to the cost of molecular testing, the variants included in tests in both research and clinical settings were often "tag SNPs" corresponding to one or more variants that were thought to be in linkage disequilibrium with the functional variant(s) [11, 12].

Several molecular techniques were utilized for early pharmacogenetic testing. In most cases, testing was limited to one gene at a time and few variants per gene. Laboratory-developed tests were offered by a small number of clinical laboratories and utilized a wide variety of techniques, including allele-specific PCR, real-time PCR, a bead-based flow cytometry platform (the Luminex xMAP system, Austin, TX, USA), a microelectronic DNA array (the eSensor, GenMark, Carlsbad, CA, USA), or other simple PCR-based techniques [13–16] (for a review of methods utilized, please see [17]). Microarray technology was also utilized, such as the AmpliChip (Roche, Pleasanton, CA, USA) test for *CYP2D6* and *CYP2C19*, which was the first FDA-approved pharmacogenetic test [18]. Another technique involved amplifying DNA surrounding a variant of interest, followed by dot blotting onto nitrocellulose squares and hybridizing the amplified DNA on each dot with a biotinylated probe specific to the normal or variant allele [19]. Although this method was labor-intensive, its potential for automation and flexibility enabled its application across multiple variants and clinical laboratory settings. As molecular techniques became more widespread, some laboratories adopted them, whereas others continued to use biochemical methods, such as TPMT activity assays; a few laboratories also developed newer alternative methods and testing strategies. For example, a method was developed that used flow cytometry to screen for *HLA-B*57:01* in the setting of HIV before the use of abacavir. The assay used a monoclonal anti-B17 antibody, and patients who tested positive with this method were then reflexed to a molecular assay specific for the *HLA-B*57:01* allele [20]. When clinical laboratories first began to offer pharmacogenetics, testing was relatively expensive, and adoption was limited. Although some methods for multiplexing many variants were available in research laboratories, the clinical test volume was initially too low to make these methods cost-effective without compromising turn-around time as large sample batches may be required. As testing volumes increased, higher throughput methods followed.

During the early years of clinical testing, *CYP2D6* was already known to be a highly polymorphic gene with copy-number variation (CNVs), including full gene deletions, duplications, and hybrid alleles resulting from recombination with the *CYP2D7* pseudogene [21–24]. Given that CYP2D6 metabolizes approximately 25% of clinically available medications, PCR-based methods to detect these important CNV events were developed [25]; however, not all laboratories testing *CYP2D6* adopted the CNV-detection methods. Some laboratories also developed methods for performing long-range PCR to fully characterize all copies of *CYP2D6* present and to isolate different forms (e.g., *CYP2D6::CYP2D7* hybrids, *CYP2D7::CYP2D6* hybrids, and the upstream vs. downstream copy of *CYP2D6* in a duplication) [26–28]. Targeted genotyping and PCR-based methods to detect CNVs were the most cost-effective approaches and could resolve most samples. The ability to isolate individual copies of *CYP2D6* using long-range PCR followed by Sanger sequencing enabled more precise genotype and phenotype calling in challenging cases [29]. Differences in CNV detection among laboratories remain today and may result in variability in genotype and phenotype assignments when CNVs are present.

CURRENT TESTING

As pharmacogenetics gained acceptance, interest grew in offering panel testing. Panels that tested multiple genes simultaneously could be useful in several scenarios: to optimize medical therapy for patients on multiple medications, when deciding the most appropriate medication when several medications metabolized through different pathways are reasonable options, or for pre-emptive testing where the results could be used for a current medication question or to guide future medications. Some early panels involved simply running individual gene tests and combining the results into a single report [30]. As molecular testing technology advanced, simultaneous testing of multiple genes became feasible and facilitated the development of cost-effective panels. Current methods include mass spectrometry (e.g., Mass Array), highly multiplexed real-time PCR using array-based technology, microarrays, and next-generation sequencing (NGS). In general, each laboratory selects the most suitable platform for its workflow. The anticipated test volume, turnaround-time requirements, cost of testing, gene(s) included, and instrumentation are all important variables that impact clinical laboratory decision-making. Some genes, such as those within the HLA locus (e.g., *HLA-A* and *HLA-B*) and *CYP2D6*, are particularly challenging to analyze and may require the use of a separate assay [29, 31]. Notably, modern genotyping and short-read NGS techniques often cannot be used to determine the phase of multiple variants within a sample. Laboratories must develop expertise in pharmacogenetics to accurately assign diplotypes, as these are typically inferred based on allele frequencies within the tested population. Additionally, specialized software or NGS pipelines may be required for testing genes within the HLA locus [31].

Although targeted genotyping is commonly used by clinical laboratories, these techniques are limited in that only relatively common genetic variants are detected. In addition, a subset of genes is highly polymorphic such that targeted genotyping-based approaches may exclude a substantial number of clinically impactful variants (e.g., *G6PD* and *DPYD*). At present, CNV is primarily only considered for *CYP2D6* in clinical pharmacogenomic testing. Although examples of clinically significant deletions in other genes (e.g., *DPYD*, *UGT2B17*, and potentially *CYP2B6*) have been reported and are of growing clinical interest, many of these are population-specific, and unique assays may be required for each CNV in the setting of targeted genotyping, which could be cost-prohibitive [32–36].

STANDARDIZATION EFFORTS

Laboratories that were early adopters of pharmacogenomic testing had few resources to guide their test design and reporting strategies, aside from the literature, until the Pharmacogenomics Knowledge Base (PharmGKB) was launched (Fig. 2) [37]. Each laboratory developed a system that was based on their interpretation of the literature. This situation resulted in variability in almost all aspects of testing, including terminology, gene and allele selection, the method of translation from genotype to phenotype, and reporting strategies. Given this variability, a test performed in one laboratory typically worked well and was understood by those using the results at that institution; however, the results could differ from those generated in a different laboratory. A lack of standardization was problematic, both for patients who moved and took their medical records (including laboratory test results) to different institutions and for data analysis in research settings. As pharmacogenomic testing was increasingly adopted, standardization efforts began. The College of American Pathologists launched a pharmacogenetics proficiency testing survey in 2007, which allowed laboratories to compare their results to those of other laboratories (Fig. 2) [38].

The star-allele nomenclature system, initially proposed in 1989 [39], was first applied in 1996 to *CYP2D6* [40] and later to other CYP enzyme-encoding genes [41]. Recognizing the importance of this system to the rapidly growing field of pharmacogenomics, the Human Cytochrome P450 Allele Nomenclature Database was established in 1999 by Magnus Ingelman–Sundberg at the Karolinska Institutet and made publicly accessible [42]. Later, in 2017, the Pharmacogene Variation Consortium (PharmVar) was created to continue to host and update the star-allele nomenclature [43–45]. PharmVar expanded curation to several non-CYP genes, including *DPYD*, *NUDT15*, *SLCO1B1*, and, most recently, *NAT2*. Today, several other sources of standardized star-allele nomenclature relevant to pharmacogenomics exist, including the UGT nomenclature website maintained by the Université Laval in Quebec City, Canada (http://www.pharmacogenomics.pha.ulaval.ca/cms/ugt-alleles-nomenclature/; last accessed, February 8, 2025), the TPMT nomenclature website maintained by Linköping University in Linköping, Sweden (https://liu.se/en/research/tpmt-nomenclature-committee; last accessed, February 8, 2025) [46], and the HLA nomenclature website (https://hla.alleles.org/nomenclature/index.html; last accessed, February 8, 2025) [47–49].

Around the same time, an international workgroup was created to improve the standardization of pharmacogenomic no-

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menclature. The workgroup included stakeholders from the pharmacogenomics community (Pharmacogenomics Knowledge Base [PharmGKB], Pharmacogenomics Research Network [PGRN], Clinical Pharmacogenetics Implementation Consortium [CPIC], European Pharmacogenetic Implementation Consortium [Eu-PIC], Ubiquitous Pharmacogenomics [U-PGx], European Society for Pharmacogenomics and Personalized Therapy [ESPT], International Federation for Clinical Chemistry [IFCC], and the ClinGen PGx Working Group), regulatory and governmental agencies, genetic-nomenclature committees, gene–variant databases, PGx gene-specific nomenclature committees and databases, professional societies, pharmacogenomic test developers, and clinical and research laboratories. Together, in 2016, this group published recommendations for variant nomenclature to be used when reporting test results [10]. Using Human Genome Variation Society nomenclature based on a reference genome or reference transcript (rather than legacy nomenclature) was among the recommendations of this group. PharmVar provides a simple mechanism to toggle among the various nomenclature systems to facilitate this transition.

Standardized star-allele nomenclature laid the foundation for many other standardization efforts. Although the CPIC was initially formed from the PGRN and PharmGKB to create guidance for using pharmacogenomic results [50], applying the test results is challenging when laboratories report differently. The CPIC also worked with stakeholders to propose standardized terminology, specifically to describe allele function as well as genotype-to-phenotype translation [51]. Although this terminology was generally accepted by clinical laboratories, *CYP2D6* proved to be more difficult. In the setting of one normal function allele and one nonfunctional allele, some laboratories reported the result as an intermediate metabolizer, whereas others reported this result as a normal metabolizer. The CPIC, along with the Dutch Pharmacogenetics Working Group, gathered experts in the field to come to a consensus to allow for more uniform *CYP2D6* reporting [52]. Although the prior CPIC article standardizing terms for clinical pharmacogenetic test results introduced the term “rapid metabolizer” to describe the predicted phenotype associated with the combination of a normal and increased-function allele, this term was ultimately excluded for *CYP2D6* during the consensus process for this gene.

In addition to nomenclature and genotype-to-phenotype translation recommendations, those for standardizing the selection of alleles that laboratories tested were also created (Fig. 2). The Association for Molecular Pathology (AMP) Working Group, along with other professional societies, created recommendations for selecting alleles for *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *DPYD*, *TPMT*, *NUDT15*, and warfarin-related genes [53–59]. For each gene, this group recommended a “must test” list of alleles, referred to as Tier 1, based on a known function of the allele, the availability of reference materials, an appreciable minor allele frequency in any known population, and an absence of major technical difficulties for testing. An additional list of alleles for each gene, referred to as Tier 2, was also recommended, but these alleles did not meet all the Tier 1 criteria. Typically, these recommendations focus on alleles that should be included in testing, although the *CYP2D6* recommendations also address copy-number testing. Specifically, the use of a single probe, which does not enable differentiation between hybrid alleles and true duplication, vs. multiple probes to overcome this limitation was discussed [53].

As laboratories transition to sequencing-based pharmacogenomic testing, rare variants will inevitably be interpreted and reported. Failure to develop a standardized approach swiftly may reintroduce variability, as laboratories independently develop methods for classifying and reporting rare variants.

TRANSITION TO SEQUENCING-BASED PHARMACOGENOMIC TESTING

As sequencing costs have decreased, a transition to sequencing-based approaches to pharmacogenomic testing has begun. Today, most laboratories use targeted genotyping-based approaches for PGx. However, some laboratories are beginning to use sequencing methods for PGx, either due to the consolidation of laboratory instrumentation and workflows or due to interest in interrogating rare variants. Sanger sequencing is widely used in laboratories, particularly for confirming variants or specific applications (such as *CYP2D6* after long-range PCR); however, it is impractical for multi-gene panels. Most laboratories adopting sequencing-based approaches are utilizing or considering short-read NGS, with a smaller subset considering long-read methods [60]. In addition to detecting both common and rare variants, a potential advantage of using NGS is that most diagnostic laboratories routinely perform exome and genome sequencing and have developed bioinformatic algorithms capable of detecting CNVs. The chemistry run by these laboratories already includes most genes important in pharmacogenomics, and their bioinformatic algorithms for CNVs may also be applied to pharmacogenes. Historically, CNVs were not thought to play a major role in pharmacogenomics, aside from *CYP2D6*, and most targeted-genotyping platforms are not designed to detect CNVs.

However, in recent years, large population databases and studies have revealed that large deletions and duplications are more common than previously recognized, and in many cases, they are population-specific [32–36]. A transition to sequencing with CNV calling would improve the panels available today.

Although short-read NGS works well for many pharmacogenes, limitations exist for genes that share substantial homology with pseudogenes (e.g., *CYP2D6*) as well as complex, highly polymorphic regions (e.g., HLA genes, such as *HLA-A* and *HLA-B*). These genes may require special software or algorithms for accurate genotype calling or an alternate method [29]. When multiple genetic variants are identified, determining the phase of the variants is not feasible unless they are within 100–150 bp of each other. Phasing is also generally not possible with targeted genotyping. However, long-read sequencing platforms are emerging as effective tools for resolving complex and homologous gene regions while providing the added capability to determine haplotypes definitively [60].

Given the applicability of NGS for multiple purposes, laboratories that have traditionally performed genetic testing for inherited disorders may now begin to include pharmacogenomic results in exome or genome sequencing or consider adding pharmacogenomic panels to their test menus. A potential challenge for these laboratories is that bioinformatic pipelines may incorporate filters that exclude variants over a set minor-allele frequency threshold. Because many pharmacogenomic variants are common, they could inadvertently be filtered out unless provisions are taken to specifically call these variants. In addition, converting individual variant genotypes to star alleles, diplotypes, and phenotypes requires specialized software, processes, and expertise, which may not be familiar to laboratories performing traditional hereditary genetic testing [61].

Although a major advantage of sequencing is that both common and rare variants can be detected, classified, and clinically interpreted, most laboratories that adopt NGS for PGx have limited their analysis to a predefined set of variants with known functions. Thus, rare variants are often excluded, and the advantages of sequencing are not fully realized today. Interpretation of variants that have not yet been classified by the CPIC or other expert groups can be challenging because the guidelines for hereditary sequence variant classification published by the American College of Medical Genetics and Genomics (ACMG) and AMP were not designed for pharmacogenomics [62]. The Mayo Clinic conducted a study using short-read NGS to perform clinical pharmacogenomic testing, and the results were deposited into the electronic health record for clinical use. In that study, all variants detected were classified and clinically interpreted [63, 64]. The laboratory used the ACMG/AMP guidelines with modifications, as well as professional judgment, to classify variants. Some rare variants clearly impacted protein function (e.g., single-base deletion resulting in a frameshift early in the gene sequence). In contrast, in other cases, rare variants had unknown functions and were classified as variants of uncertain significance owing to limited data. A major challenge in the clinical reporting of rare pharmacogenomic variants beyond classification was also identified. When variants that comprise a known diplotype (i.e., star alleles) are identified along with a rare variant, no system exists for clinical reporting aside from submitting them to PharmVar to create a new star allele, which does not fit the timeline required for clinical reporting. In addition, when variants of uncertain significance are encountered, the predicted phenotype is also uncertain. The Mayo Clinic laboratory developed a system for reporting both genotypes and phenotypes, including rare variants, while conveying uncertainties. This system is illustrated in Fig. 3 and detailed by Lopes, *et al*. (2022) [63]. Whether this approach will be adopted more widely or a different system will be created remains unclear.

To evaluate the importance of transitioning to sequencing-based approaches, an *in silico* panel was constructed that incorporated variants present in over 50% of current clinical pharmacogenomic tests. This panel was applied to sequencing results generated in the Mayo Clinic study. Variants were categorized into those that are commonly tested by current clinical tests and those that would remain undetected. Comparisons of genotype results revealed that approximately 28% of individuals harbored at least one rare variant that may be clinically significant and would be undetected using a typical genotyping panel [63]. *CYP2D6* and *DPYD* accounted for the highest number of unique variants not identifiable by current panel-based approaches. Expanding genotyping panels to gain equivalency to sequencing-based approaches would be impractical, given the sheer volume of unique variants.

Despite challenges in variant classification, the importance of rare variants is becoming increasingly recognized. As sequencing costs continue to drop, the field will likely transition to sequencing-based approaches in the coming years. These changes may paradoxically lead to a step backward in standardization for several reasons: (1) no framework currently exists for classifying rare pharmacogenomic variants detected during routine clinical testing; (2) as indicated earlier, no system exists for reporting rare variants along with star alleles; (3) each laboratory may have different preferences for including or excluding rare vari-

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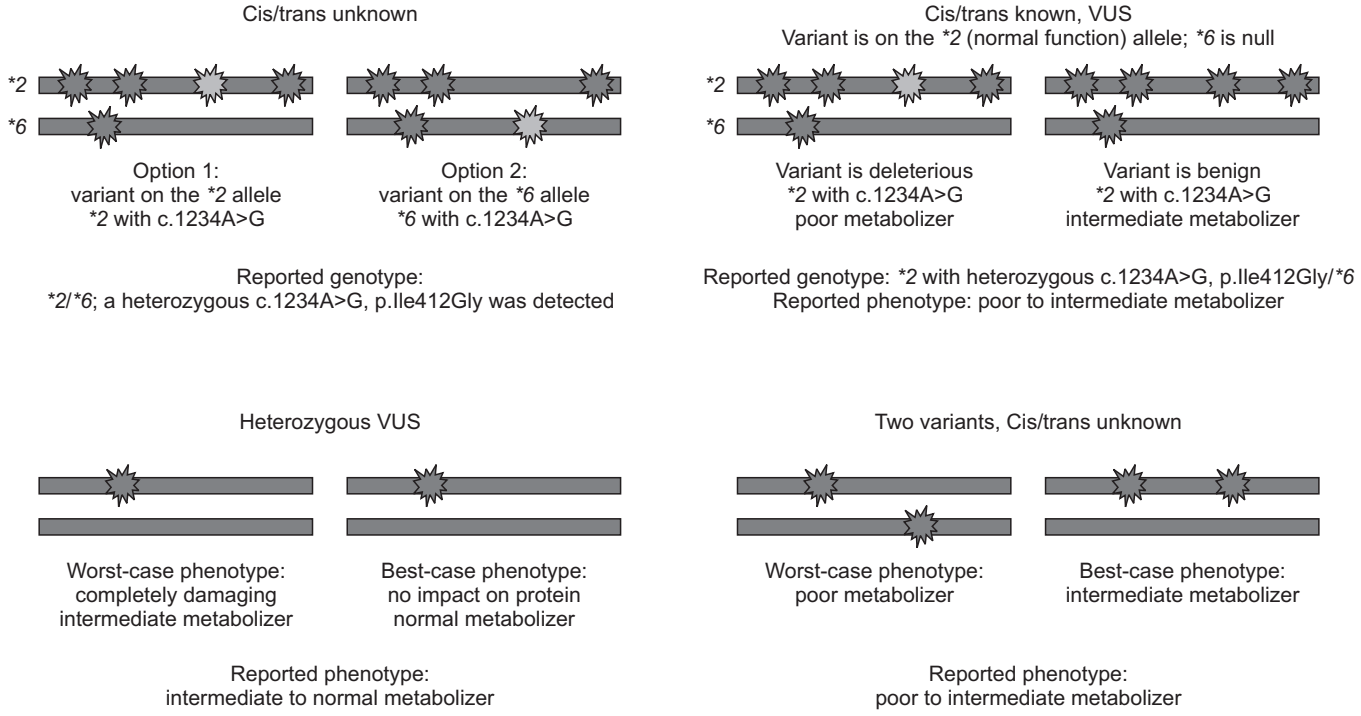


Fig. 3. Example of a nomenclature system incorporating rare variants into the star-allele system. The figure presents four examples of different scenarios. The jagged symbols indicate genetic variants, whereas the lines indicate individual alleles. Reprinted from the *Journal of Molecular Diagnostics* [63], Copyright 2022, with permission from Elsevier.

ants or variant types; and (4) there are no standard reporting guidelines for laboratories including PGx results as an add-on to diagnostic exome or genome sequencing. Efforts to develop a framework of tiered standard terminology for gene–drug response validity and definitions for classifying PGx genes and variants are underway by the newly established Clinical Genome Resource (ClinGen) Pharmacogenomics Working Group (PGxWG) [65]. In terms of variability in reporting, laboratories should be encouraged to increase transparency in their reports by including the variants that were detected, as well as information regarding the pharmacogenes and variants or regions of interest that were included in the test. For example, some laboratories may choose to limit analysis to only variants with a known function, other laboratories may also classify and report variants with a clear loss-of-function (i.e., non-sense variants or frameshifts), and others may choose to interpret all variants detected using their system or a modified form of the ACMG/AMP system [62]. Notably, in pharmacogenomics, negative results are also clinically significant. Excluding results for pharmacogenes with no detected variants from reports can create ambiguity regarding whether the gene was tested and no variants were found or the gene was not interrogated. Developing guidance for laboratories for reporting pharmacogenomics in the context of diagnostic exome or genome sequencing may help maintain the progress made in standardization.

FUTURE PERSPECTIVES

Pharmacogenomic testing in clinical laboratories has rapidly evolved and will undoubtedly continue to progress as the underlying science advances. A summary of the advances we anticipate in the coming years is described in this section and highlighted in Table 1. Currently, the functional consequences of relatively common variants in coding regions of genes are understood, whereas rare and non-coding variants are not well-characterized. Clinically, many rare and non-coding variants are typically not considered, partly owing to the lack of a system to evaluate these variants, as well as limited data. Although *in silico* tools to predict changes in splicing and the impact of amino acid changes exist, they are not robust enough to be used in isolation [66, 67]. Other variables, such as evolutionary conservation and the minor allele frequency (which are used to evaluate germline variants in the context of disease), may not be applicable due to a lack of selective pressure against deleterious variants in drug-

Table 1. Future opportunities for clinical laboratory pharmacogenomics

Future opportunities for pharmacogenomics	
Transition to sequencing and detection of rare variants	Methods to classify variants as clinically encountered CNV detection across pharmacogenes Phasing by using long-read sequencing
Advances in variant classification	Improved *in silico* tools using artificial intelligence High-throughput functional studies (MAVE analysis) Inclusion of non-coding variants and epigenetics
Drug–response predictions using many genes	Combinatorial pharmacogenomics Polygenic risk scores Increased focus on pharmacodynamic genes

Abbreviations: CNV: copy-number variation; MAVE: multiplexed analysis of variant effects.

metabolizing enzymes. Functional studies are particularly important in pharmacogenomics. Functional studies have traditionally been quite labor-intensive, but newer high-throughput techniques, referred to as either deep mutational scans or multiplexed analysis of variant effects (MAVEs), have been developed and are starting to be applied to pharmacogenomics [68–71].

Large data sets that provide functional data for many variants within a gene (generated using MAVE analysis) can also be utilized to improve *in silico* prediction tools. Early tools for predicting the impact of missense changes relied on biochemical differences between amino acids, evolutionary conservation, or patterns of genomic constraint. Currently, proteome-wide predictions of structural changes and stability are beginning to become incorporated, as is the use of artificial intelligence to identify patterns and assess data [72, 73]. The lack of large data sets of functionally characterized variants across many genes has been a limitation; however, MAVE analysis with additional genes will provide more training data and improve these algorithms, perhaps facilitating increased adoption of sequencing-based pharmacogenomic testing methods along with the interpretation of rare variants.

Non-coding variants may impact splicing, transcription, RNA stability, RNA processing, translation, and chromatin interactions across loci, and while several known non-coding variants are routinely included in pharmacogenomic tests (i.e., *DPYD* c.1129-5923C>G; *VKORC1* c.-1639G>A), most are excluded. The challenge of classifying non-coding variants is not unique to pharmacogenomics and is increasingly recognized in germline diagnostic testing as genome sequencing is adopted. Recommendations were recently published for the clinical classification of variants in non-coding regions [74]. Most recommendations provided may be applicable to pharmacogenomics; however, investigating the underlying biology of non-coding regions and developing tools to predict the impact of variants in these regions is essential for effectively interpreting the impact of non-coding variants on drug–response phenotypes. These challenges are not unique to non-coding variants. Similarly, epigenetic variation also contributes to variable drug responses and may alter coding or non-coding regions [75]. Pharmacoepigenetics is an area of active research; however, limited data and potential tissue-specific epigenetic modifications are barriers to clinical application. As this field advances, it will likely be incorporated to at least some degree into clinical testing to further refine drug–response predictions.

Although most clinical pharmacogenomics currently involves testing for one or a few genes to predict the toxicity or efficacy of a drug, the associated metabolic pathways are much more complex. Combinatorial pharmacogenomic algorithms have been developed to account for the contributions of multiple enzymes. However, in many cases, these algorithms have been largely theoretical and are often proprietary to a specific commercial laboratory. Some algorithms have been supported by clinical studies, whereas others have not, and these algorithms might not provide consistent results [76, 77]. Despite the limitations of some algorithms, the concept that variation across multiple genes with smaller effect sizes may be important in addition to the commonly tested single gene–drug pairs with large effect sizes is generally accepted. Polygenic risk scores (PRSs) have begun to emerge for inherited disorders and pharmacogenomics.

In many cases, PRSs have been developed for a disease, followed by an attempt to apply them to pharmacogenomics; however, pharmacogenomic-specific methods may be preferable [78, 79]. PRSs are also difficult to create for pharmacogenomics due to the paucity of large data sets that include detailed information with pharmacological outcomes. Additionally, many data

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sets include patients with polypharmacy and/or underlying disorders, which adds complexity [80]. Furthermore, existing data are biased toward individuals of European ancestry, which limits the generalizability and implementation for other populations [80]. Despite these limitations, ongoing research may lead to the development of more robust PRSs that will ultimately become incorporated into clinical testing in the coming years.

Most pharmacogenes commonly tested today are part of pharmacokinetic pathways, with few examples of routinely tested genetic variants in pharmacodynamic pathways (e.g., *VKORC1*). In the future, pharmacogenomic testing may merge with diagnostic testing for inherited disorders as genetic variation in pharmacodynamic targets is identified and found to be important for drug selection. Lumacaftor–ivacaftor combination therapy, which is used to treat cystic fibrosis, represents an early example [81]. Lumacaftor acts as a chaperone that facilitates folding and processing to allow more of the otherwise misfolded F508del CFTR protein to be expressed on the cell surface, whereas ivacaftor serves as a potentiator that can keep the CFTR channel open. While this combination is used for individuals homozygous for the F508del variant, ivacaftor is effective for several pathogenic variants. A more recent example involves monoclonal antibody drugs targeting amyloid beta (e.g., aducanumab and lecanemab) that are used for Alzheimer's disease [82, 83]. These medications include a boxed warning indicating that individuals homozygous for the *APOE e4* haplotype may have a higher incidence of amyloid-related imaging abnormalities (ARIAs). ARIAs are usually asymptomatic, but serious and life-threatening events can occur on rare occasions. The warning indicates that *APOE* testing should be performed before initiating therapy to inform the risk of developing ARIAs. Casimersen, which is used to treat individuals with Duchenne muscular dystrophy and mitigates the effects of pathogenic variants by promoting exon 45 skipping, is another example [84]. Although these are examples of pharmacogenomics involving pharmacodynamic genes, the inclusion of *CFTR*, *APOE*, and *DMD* in pharmacogenomic tests is not practical as most patients do not have cystic fibrosis, Alzheimer's disease, and/or Duchenne muscular dystrophy. Instead, this information would come from a diagnostic test for cystic fibrosis or muscular dystrophy or a test specifically for *APOE*, which may also be used to guide medication therapy. As medicine continues to advance, genetic variation in pharmacodynamic genes is anticipated to be leveraged to create more specific, targeted therapies to help patients. As pharmacogenomic testing transitions to sequencing-based methods, the lines between diagnostic testing and pharmacogenomics may blur with both being reported simultaneously.

CONCLUSIONS

Clinical pharmacogenomic testing has a strong foundation. Today, the field is prepared for a rapid expansion that will be necessary to accommodate the testing of an increasing number of patients as clinical adoption continues. In addition to the anticipated growth of existing testing, a transition to sequencing, adoption of PRSs, incorporation of epigenetics, and a larger focus on pharmacodynamic targets are on the horizon. Ultimately, the success thus far of pharmacogenomics has been due to knowledge sharing between research groups, clinical practitioners, and clinical laboratories. Continued knowledge-sharing and standardization efforts will be necessary to make the dream of personalized medicine through pharmacogenomics for all patients a reality.

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AUTHOR CONTRIBUTIONS

Moyer AM and Black JL contributed to the conception and design of the study. Moyer AM wrote the original draft. Moyer AM and Black JL edited the manuscript.

CONFLICTS OF INTEREST

Moyer AM has no conflicts of interest to declare. Black JL declares intellectual property and royalties with AssureX Health (Myriad) and stock, royalties, and intellectual property with Oneome LLC.

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