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Comparative Insights into NGS Platforms for Clinical Pharmacogenomics: Advantages, Limitations, and Workflow Strategies

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ABSTRACT

Pharmacogenomics (PGx) focuses on using genetic information to improve and personalize drug therapy. Many clinical centers have already begun incorporating pharmacogenetic testing into routine care. Next-generation sequencing (NGS) is becoming a more comprehensive, efficient, and cost-effective tool for PGx applications. This review outlines the key factors involved in using NGS to guide medication decisions in clinical settings. It examines both the benefits and the challenges associated with adopting NGS-based PGx testing. In addition, it describes the limitations of different NGS platforms and highlights practical strategies for establishing and managing these technologies in clinical practice.

Keywords: Clinical implementation, Pharmacogenomics, Next generation sequencing, Clinical practice, PGx testing

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Introduction

The growing significance of pharmacogenomics in healthcare

Pharmacogenomics (PGx) focuses on how a person's genetic makeup can influence their response to medications, helping identify individuals who may face a higher risk of side effects or poor drug efficacy. Research has shown that many drug-related genes, known as "pharmacogenes," contain a wide range of functional genetic variations (FGVs), and different gene variants can lead to varied treatment outcomes [1-3]. Studies indicate that nearly all individuals—around 97–98%—carry at least one FGV that may affect drug response, and the chance of having a loss-of-function (LOF) variant in a pharmacogene is about 93% [4]. Recognizing these genetic differences is therefore essential for choosing the most effective dose and medication while minimizing unwanted reactions or treatment failure.

To help clinicians interpret PGx findings, several organizations provide structured guidelines. The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have created extensive, evidence-based recommendations for numerous gene–drug pairs [5, 6]. CPIC was formed in 2009 through a collaboration between PharmGKB and the Pharmacogenomics Research Network (PGRN), while DPWG began in 2005 under the Royal Dutch Pharmacists Association. Their guidance, together with FDA drug–gene interaction updates, supports the translation of PGx results into practical prescribing decisions. Many certified laboratories around the world now offer PGx testing, and available tests can be browsed through the Genetic Testing Registry (GTR, https://www.ncbi.nlm.nih.gov/gtr/) [7].

Introducing next-generation sequencing (NGS) into PGx represents an important shift for the field. Rather than ordering individual gene tests only when needed, healthcare systems are moving toward broader, pre-emptive genotyping that evaluates multiple genes involved in drug absorption, distribution, metabolism, and excretion

(ADME) using various NGS technologies [8, 9]. This approach generates a full set of PGx-relevant variants that can be used to predict patient phenotypes and guide medication and dosing decisions **(Figure 1)**.

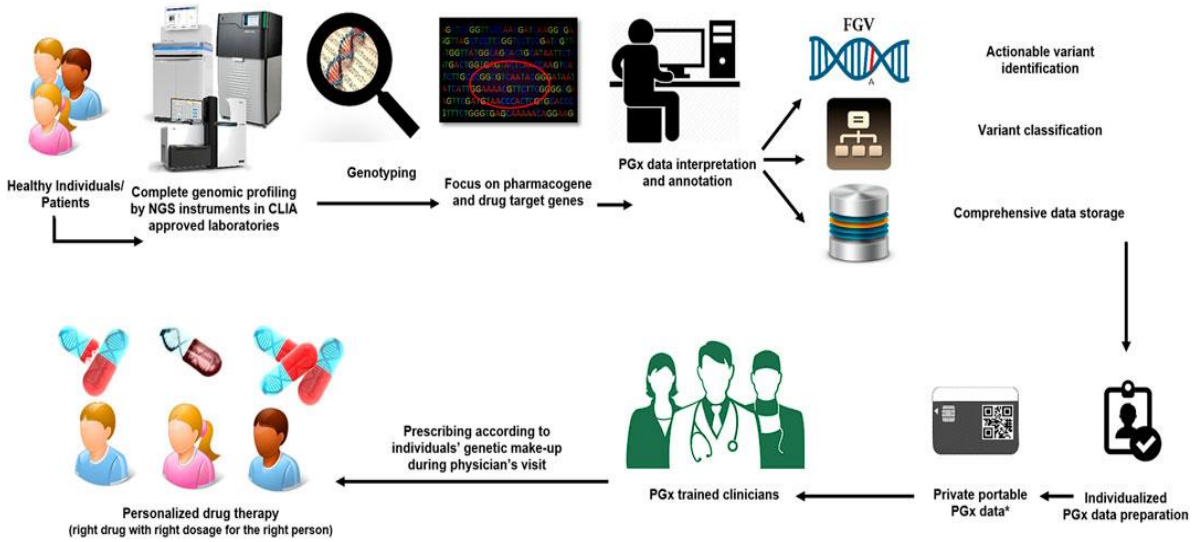

Figure 1. illustrates a future scenario in which pharmacogenomics becomes a standard part of medical care. In this model, every individual—whether healthy or ill—would undergo broad genomic screening before meeting with a clinician. Using specialized bioinformatics tools, genetic variants across all relevant pharmacogenes would be annotated and visualized. Each person's results would then be stored on a secure, portable PGx electronic card. Clinicians trained in pharmacogenomics could use this personalized genetic profile to select the most appropriate therapies and dosages for each patient.

Although the application of next-generation sequencing (NGS) in PGx has been reviewed previously [10], this article focuses more specifically on the difficulties associated with detecting certain variant types and the complexities involved in interpreting these findings in a clinical setting. It also outlines practical considerations for implementing and managing NGS technologies in healthcare environments. Several detailed tables presenting key NGS-related PGx information are provided, and a brief glossary of sequencing terminology is included in Appendix 1 for clarity.

How NGS can be applied in PGx

This section begins with an overview of SNP-based PGx testing, which remains the most commonly used approach in routine clinical profiling. It then describes more advanced methods, including targeted sequencing and whole-exome or whole-genome sequencing (WES/WGS).

SNP-based PGx testing in clinical care

Rapid, reliable, and low-cost genotyping technologies are central to expanding PGx use in medicine. At present, clinical laboratories typically rely on SNP-based methods such as real-time PCR with TaqMan probes, restriction fragment length polymorphism (RFLP) assays, and fixed gene panel tests like ADME arrays [11-16]. Genome-wide arrays, such as the Infinium Global Screening Array (GSA), could theoretically be used for PGx screening, but they are not yet widely adopted. Their current limitations include poor detection of structural variants—such as copy-number variations (CNVs), hybrid alleles, and CYP2D6/7 rearrangements—and restricted coverage of pharmacogene alleles, focusing mainly on common or previously identified variants. Although newer arrays now incorporate thousands of drug-related markers [17-19], they do not provide haplotype-level phasing, which can hinder accurate phenotype prediction.

These limitations make NGS technologies increasingly appealing for clinical PGx applications. Over recent years, multiple studies have assessed the utility of targeted sequencing, WES, and WGS for pharmacogenomic testing. **(Table 1)** summarizes several of these investigations according to the sequencing strategies used.

Table 1. Summary of Next-Generation Sequencing (NGS) Studies in Pharmacogenomics: Platforms, Scope, and Key Findings

Study Objective	Sample Size	NGS Platform(s) Used	Genes / Variants Covered	Key Findings / Conclusion	Reference
Platform validation & variant discovery	3 × 96 = 288	Targeted sequencing (PGRNseq panel)	84 pharmacogenes, focus on SNVs	Custom PGRNseq panel highly suitable for detecting common and rare PGx variants in large cohorts and clinical use	[20]
Platform validation & variant discovery	376	Targeted sequencing	114 core ADME genes, SNVs	Ready-to-use targeted panels enable comprehensive PGx profiling including rare variants for personalized medicine	[21]
Platform validation	2 (cell lines)	Targeted sequencing	3 challenging ADME genes (SNVs, CNVs, InDels)	Accurate detection of complex variants and haplotypes in difficult ADME genes	[22]
Platform validation & variant discovery	235	Targeted sequencing (PGxSeq panel)	39 genes, 100 SNVs + CYP2D6 CNV, UGT1A1*28	High-accuracy panel successfully identifies clinically actionable variants and complex structural alleles	[23]
Platform validation & variant discovery	150 (Caucasian liver donors)	Targeted sequencing	340 ADME genes, >7,000 novel variants	>99% accuracy; functional prediction enables prioritization of novel variants	[24]
Validation of known variants	60	Targeted sequencing	20 SNVs & InDels	Sequencing data accurately predicted atorvastatin plasma concentrations	[25]
Platform comparison & validation	98	Targeted sequencing + WGS	19 SNVs + CYP2D6 CNVs	>97% concordance between platforms; 95% of children carried ≥1 actionable PGx variant	[26]
Validation of known variants	1,583	Whole-exome sequencing (WES)	11 pharmacogenes, actionable phenotypes	86% of individuals had ≥1 actionable PGx phenotype; WES repurposing useful for 7/11 key genes	[27]
Validation of known variants	94	Whole-exome sequencing	CYP2C19, CYP2C9, VKORC1 (3 SNVs)	91% carried relevant variants; 20% had immediate implications for current medication	[28]
Platform validation	36 + 12	Whole-exome sequencing + amplicon seq	36 SNVs & InDels	High concordance (>99%) with other platforms; WES is a promising low-error tool for PGx profiling	[29]
Platform & CNV discovery validation	2,504 (WGS) + 59,898 (WES)	WGS + WES	208 SNVs + population-specific CNVs	Revealed deletions/duplications in 97% of subjects, confirmed by Sanger	[30]
Variant discovery	1,000 Genomes Project data	Whole-genome sequencing	160 potentially functional SNVs	Identified putative causal variants within known PGx loci	[31]
Variant validation & discovery	547 + gnomAD	Whole-genome sequencing	11 pharmacogenes, SNVs & InDels	WGS is the most comprehensive and feasible approach for precision PGx testing	[32]
Platform comparison	~44,000 biobank participants	WGS, WES, microarray	11 key pharmacogenes + CNVs	WGS and microarray show highest concordance; WES not ideal; PGx implementation could affect ≥50 doses/1,000 people	[33]
Variant discovery (anti-TNF response)	3	Targeted sequencing	16 SNVs	Rapid, cost-effective NGS method identified variants impacting anti-TNF response	[34]
Variant discovery	392	Whole-exome sequencing	~21,000 SNVs	Revealed novel loci strongly associated with clopidogrel response and platelet reactivity heritability	[35]

(platelet reactivity)					
Variant discovery	482 + 7	Whole-genome sequencing	231 pharmacogenes, >17,000 ADME variants	Detected 1,012 novel potentially deleterious variants across exons, introns, and regulatory regions	[36]
Variant discovery	100	Whole-genome sequencing	437 SNVs (227 common, 466 rare)	Population-specific functional SNVs identified in Indian cohort	[37]

Targeted sequencing panels

Years of pharmacogenomics (PGx) research have uncovered many genes involved in drug metabolism, transport, and action. However, only a subset of these genes shows strong, well-validated links to drug response outcomes. As a result, CPIC and DPWG limit their clinical dosing recommendations to specific variants within established pharmacogenes.

To support both clinical use and discovery research, Gordon *et al*. designed the PGRNSeq panel, aiming to achieve an optimal balance between sequencing depth, sample capacity, and overall cost. Their panel incorporated CPIC-annotated genes along with additional genes suspected to play a role in PGx. Although the panel performed well, the authors noted that improved methods are still required for analyzing non-coding regions and complex structural variants in genes such as CYP2A6, CYP2D6, and HLA-B, as well as for enhancing computational interpretation [20]. Similarly, Han *et al*. created a broad NGS-based panel and concluded that comprehensive panels of this type can be powerful tools for investigating PGx genes, selecting candidate genes largely from the PharmaADME database [21].

Custom PGx panels have also shown high accuracy in detecting variants relevant to clinical testing. Gulilat *et al*. introduced PGxSeq, a targeted exome panel designed to capture both SNVs and CNVs in key pharmacogenes. Their findings supported PGxSeq as a reliable method for detecting both known and novel variants, although the panel was validated on a limited number of loci—39 positions across 16 genes—and did not cover regulatory or non-coding regions [23]. In another effort, researchers in Germany developed a large PGx panel covering coding regions, nearby intronic sequences, and untranslated regions (5′ and 3′ UTRs) of 340 ADME-related genes. Gene selection was based on PharmaADME, PharmGKB, and published literature. The panel showed >99% accuracy and allowed the detection of both common and rare variants, including functionally relevant non-coding changes. However, limitations included incomplete detection of certain InDels, challenges in interpreting rare variants with available prediction tools, and constraints related to sample size [24].

Long-read sequencing for PGx gene panels

Many pharmacogenes contain complex genomic features—such as pseudogenes, duplications, and tandem repeats—that are difficult to analyze accurately with short-read sequencing. Long-read technologies, which can generate reads exceeding 10 kb, have already been used successfully to characterize similarly complex loci and are now being explored for PGx applications [27, 38, 39].

Ammar *et al*. used long-read sequencing to characterize variants and haplotypes in three pharmacogenes known for structural complexity: CYP2D6, HLA-A, and HLA-B. The haplotypes they generated were validated by HapMap datasets and by phasing data from Complete Genomics WGS studies, along with Sequenom genotyping for CYP2D6. Their results demonstrated that long-read technologies can effectively resolve complex PGx variation [22]. Beyond haplotyping, phasing itself remains a major challenge in PGx. To address this, Liau *et al*. applied the GridION platform to sequence and phase the entire CYP2D6 gene. Their approach accurately identified known and novel alleles, including duplicated alleles, while simultaneously processing multiple samples. The method proved both time-efficient and cost-effective, highlighting its promise for clinical PGx genotyping [40].

Whole-exome sequencing

Broad sequencing approaches such as whole-exome sequencing (WES) and whole-genome sequencing (WGS) can detect large numbers of pharmacogenetic markers and may also support the identification of previously unrecognized loci. Although WES can be repurposed to assess known PGx variants, interpreting newly discovered variants remains difficult because functional consequences often require follow-up studies or detailed in-vitro validation. This is especially challenging when the effect of a variant on protein activity or expression is uncertain.

Van der Lee *et al*. examined whether existing WES datasets could be used to extract a PGx profile consisting of 42 variants across 11 pharmacogenes. Using the Ubiquitous Pharmacogenomics (U-PGx; www.upgx.eu) panel—based on all actionable variants listed in DPWG guidelines—they were able to retrieve 39 of the 42 variants. An actionable PGx phenotype was identified in 86% of participants. Despite the inability to detect structural variants and copy-number changes in genes such as CYP2C19, UGT1A1, CYP3A5, and CYP2D6, and despite limitations in gene number and sample size, the authors concluded that WES can generate clinically meaningful profiles for 7 of the 11 pharmacogenes examined [41].

To further assess the value of clinical WES data for secondary PGx analysis, Cousin *et al*. reviewed WES results from patients to identify functional genetic variants (FGVs) in three key pharmacogenes. These findings, combined with each patient's medical record, were interpreted by a pharmacist using CPIC, UpToDate, Micromedex, and AskMayoExpert resources. The study suggested that PGx information obtained early in life could guide safer and more effective prescribing in the future [28].

Wee Chua *et al*. evaluated the accuracy of WES-based variant calling by comparing WES results with MiSeq amplicon sequencing and with the iPLEX ADME PGx panel in separate cohorts of 36 and 12 samples. Concordance rates reached 99% in both comparisons, indicating that WES offers high reliability for PGx profiling, with an estimated error rate below 1% [29]. Nonetheless, WES has important gaps: several clinically relevant variants—including CYP2C19*17 and VKORC1—fall outside standard exome capture regions.

Whole-genome sequencing

WGS offers a complete survey of an individual's genomic variation, including all PGx-related markers. Although the interpretation of such large datasets is still complex, declining sequencing costs and the comprehensive nature of WGS may eventually make it the preferred platform for clinical pharmacogenomics.

Using WGS data from phase 1 of the 1000 Genomes Project, Choi *et al*. performed extensive annotation and identified 69,319 variants across 160 pharmacogenes—127 CPIC genes and 64 VIP genes from PharmGKB. Of these variants, 94% were SNVs and 6% were InDels. More than 8,000 variants showed strong linkage disequilibrium ($r^2 > 0.8$) with known PGx markers, and variants were distributed across intronic, coding, and upstream/downstream regulatory regions. The authors highlighted numerous potentially functional variants that may influence drug response and emphasized that direct genotyping is preferable to relying on LD patterns, which vary between populations. Reported limitations included modest sample size, exclusion of rare variants (MAF < 0.01), and the absence of experimental validation. Even so, these findings demonstrate how WGS-based PGx research can support translation of genomic insights into clinical practice [31].

While established PGx gene panels can be extracted from WGS datasets for use in prescribing, the remaining genomic information provides a valuable resource for additional discovery-driven PGx studies.

The number of functional CNVs in ADME genes is very different among people from different populations [42, 43]. NGS data can also help detect these CNVs in many ethnic groups. Researchers used WGS and WES information from the 1,000 Genomes and ExAC databases to study CNVs in 208 pharmacogenes. They found many new CNVs, including deletions in 84 percent of the genes and duplications in 91 percent of them. These results came from six population groups: non-Finnish Europeans, Africans, Finns, East Asians, South Asians, and admixed Americans.

The study showed that full NGS-based genotyping is necessary to detect CNVs and understand how common they are in different populations. Scientists can also study how these CNVs influence drug responses by looking at rare variants within each group [30].

Using NGS to find actionable variants in a person's genome may allow their PGx information to be useful throughout their lifetime. In the future, new bioinformatics tools may also help re-analyze NGS data and predict the function of newly discovered variants [28].

Targeted sequencing works well when the goal is to test known PGx genes, even those with rare variants. However, if the goal is to discover new pharmacogenes, WGS and WES are better choices [33]. WES and WGS also allow doctors to reuse existing sequencing data and extract PGx profiles for guiding drug treatment. Even though NGS platforms have many benefits, several challenges still exist. These challenges are explained next.

Challenges in Using NGS to Decode PGx Variants

Studies show that NGS can detect many types of variants in both coding and non-coding parts of drug-related genes. These include SNVs, InDels, CNVs, and some structural changes. This capability improves further when long-read sequencing and WGS are used. Still, some important pharmacogenetic variants remain hard to detect.

One major example is the CYP2D6 gene. This gene has more than 100 known alleles across different populations (www.pharmvar.org). The gene is difficult to analyze because it has very high similarity to nearby pseudogenes such as CYP2D7 and CYP2D8. There are also many recombination events and structural changes in this region, and CNVs are very common. Because of these issues, short-read NGS cannot clearly define a person's CYP2D6 profile, which makes phenotype prediction difficult. The alignment process also becomes complicated, and this makes clinical interpretation harder.

High-resolution long-read sequencing can solve some of these problems. However, long-read devices with low error rates, such as PacBio Sequel HiFi II, are only available in highly specialized centers. They are not used in routine clinical practice [44]. These platforms are also not considered suitable for large-scale PGx studies at this time [27].

Another challenging gene is UGT1A1. This gene carries important variants in its non-coding regions, including TA repeats in the promoter, such as UGT1A1*28. These repeats affect transcription and enzyme activity [45-47]. The gene contains more than 113 functional variants. Many of these variants increase or decrease enzyme activity, while others have unknown effects. The frequency of these variants depends strongly on the population.

Most genotyping panels only include common variants, which means important predictive variants can be missed. For example, the FDA approved testing for the *28 allele in irinotecan treatment but did not approve testing for the *6 allele. However, the *6 allele is the main cause of reduced UGT1A1 activity in Asian populations [48].

WES also has weak coverage in non-coding regions. As a result, its concordance is lower, and diplotype and CNV calls for UGT1A1 are often inaccurate [41].

Another challenging set of genes in pharmacogenomics is the HLA region. These genes are highly similar in sequence, which increases the risk of errors during capture and alignment in sequencing. With over 21,000 alleles, multiple pseudogenes, and intronic InDels in both class I and class II HLA genes, analyzing this region requires specialized sequencing platforms and advanced bioinformatics pipelines, particularly for populations that have not been well studied [49].

HLA variants are clinically relevant not only in drug response but also in broader areas like organ transplantation and the study of complex diseases. However, most HLA variants are rare, population-specific, and are not routinely assessed in clinical PGx testing [50]. Although various computational tools now exist for HLA variant calling and haplotype phasing using WGS, WES, or targeted sequencing data, these tools typically require high sequencing coverage for reliable allele imputation [51]. Reviews of available HLA analysis software and their strengths and limitations have been published [52-54].

Successfully interpreting PGx variants in difficult genes demands that physicians ordering tests are familiar with the latest pharmacogenomic knowledge. Testing centers must select sequencing technologies and analysis tools appropriate to each gene and patient population. Some alleles also show substrate-specific effects, which must be considered in clinical interpretation. For instance, CYP2D6*17 produces an enzyme that metabolizes haloperidol more efficiently but is less effective for codeine metabolism [55, 56]. Additionally, differences between guideline recommendations for assigning genotypes to metabolic phenotypes can complicate therapeutic decisions [57]. **(Table 2)** summarizes several of the most technically challenging pharmacogenes and the key considerations needed for sequencing and panel design.

Genes presenting major technical challenges for accurate pharmacogenomic testing

Table 2. Pharmacogenes with the associated challenges that render them difficult to genotype.

Gene	Key Challenge(s)	Reference(s)
CYP2D6	– Complex structural variants and gene rearrangements	[58]
	– Presence of pseudogenes (CYP2D7, CYP2D8)	PharmVar CYP2D6 structural variations
	– Copy-number variations (deletions, duplications, multiplications)	
	– High number of novel/rare variants	
	– Highly polymorphic and homologous region	
	– Substrate-specific phenotypic effects of certain alleles	
UGT1A1	– Rare population-specific variants	[59]
	– Clinically relevant variants located in non-coding/promoter regions (e.g., *28 TA-repeat)	[60]
	– Independent haplotypes with weak linkage disequilibrium	

VKORC1	– Most important warfarin-sensitivity variants located in non-coding regions	[61, 62]
HLA	– Extremely high polymorphism	[49]
	– Numerous rare, population-specific risk alleles (e.g., drug hypersensitivity)	[63]
SLC6A4	– Rare population-specific functional variants (e.g., 5-HTTLPR, rs25531)	[64]

Challenges and opportunities in NGS data analysis and interpretation

Interpreting NGS data for pharmacogenomics, particularly for predicting drug response phenotypes, is a highly specialized task that requires both molecular and clinical expertise. Extracting actionable, potentially pathogenic, or likely pathogenic variants from large and complex raw datasets demands significant time, careful validation, and robust computational methods. Current approaches include specialized PGx tools for star allele calling in pharmacogenes, which will be discussed in the following sections. Here, we focus on the main considerations, highlight features of commonly used PGx analysis tools, and suggest strategies to address these challenges.

Targeted sequencing panels

Unlike standard genotyping methods, sequencing provides the opportunity to identify novel variants within the sequenced regions. This has been observed even in targeted panels of well-known pharmacogenes, where previously unreported variations were discovered alongside common markers [23]. Variants of unknown clinical significance (VUS) present a key challenge for clinical implementation because their functional impact on drug response is unclear. Correctly interpreting VUS is critical, as ignoring them could result in true functional alleles being considered non-actionable.

Because targeted panels typically generate fewer VUS, replication and validation are more feasible. Approaches such as orthogonal genotyping, in silico predictions, screening of first-degree relatives, and meta-analysis using datasets like GWAS, HapMap, or gnomAD can help determine whether a VUS is likely neutral or associated with a pharmacogenomic phenotype [65].

Whole-exome and whole-genome sequencing

VUS are more common when using WES or WGS, and interpretation becomes even more complex when novel pharmacogenes are involved. Various computational tools—such as SIFT, PolyPhen2, CADD, PROVEAN, and ensemble tools like VEP and REVEL—can predict the potential functional impact of variants. However, these algorithms mainly rely on evolutionary conservation and sequence alignment, which are often less informative for pharmacogenes. Recent studies have shown that these tools may have limited predictive value for drug-related genes [66, 67].

High-throughput sequencing can also reveal incidental findings (IFs), also called secondary findings according to ACMG guidelines [68]. These are functional variants in drug-related genes that were not specifically targeted but may influence drug efficacy or the risk of adverse drug reactions. Proper interpretation of such findings requires rigorous genotype-phenotype correlation studies, validated genomic assays, and access to curated PGx resources [69].

The occurrence of secondary findings is also influenced by technical aspects of NGS, such as coverage depth, sequencing methodology, and sample selection, including family members or randomly selected patients [70]. Not all secondary findings need to be reported in clinical tests. ACMG provides guidance for reporting certain secondary findings in clinical sequencing, but these policies mainly pertain to disease-causing variants rather than pharmacogenes [71, 72]. Many pharmacogenetic variants are not associated with disease, making their reporting less clear, especially when only a targeted set of genes is tested.

Nonetheless, the goal of PGx testing is to comprehensively profile genes that may affect drug response. When using untargeted methods such as WES or WGS, it may be most appropriate to consider all identified variants as potentially relevant for future therapy, rather than designating some as secondary findings.

Recently developed bioinformatics tools for PGx variant detection

Significant efforts have been made to develop bioinformatics tools specifically for pharmacogenomics, designed to detect SNVs, CNVs, structural rearrangements, gene deletions, duplications, haplotype phasing, diplotype calling, and phenotype prediction from NGS data in clinical settings. Tools such as Stargazer, PharmCAT, Astrolabe, Aldy, and CyriPI employ specialized algorithms to interpret PGx variants accurately [47, 66, 73-75]. Additionally, tools like g-Nomic and PHARMIP provide clinical recommendations based on the results of PGx

tests [76, 77]. A summary of the advantages and limitations of these tools has been described previously [78], and **(Table 3)** offers a concise overview of their key features.

Among the most extensively used tools, Stargazer, Astrolabe, and Aldy have been systematically evaluated. Twesigomwe and colleagues conducted a comparative study focused on calling CYP2D6 variants. They found that Aldy and Astrolabe outperformed Stargazer in identifying both common and rare SNVs. Stargazer, however, showed better performance in phasing rare homozygous alleles due to a built-in supplementary algorithm. All three tools faced challenges in calling InDel star alleles and hybrid rearrangements from short-read NGS data. For structural variants, including gene deletions, duplications, and multiplications, Aldy achieved higher concordance than Stargazer and Astrolabe. Astrolabe, in contrast, demonstrated weaker performance in structural variant detection. While Stargazer excelled in CNV detection and hybrid rearrangement identification, it also reported the highest number of non-genotyped diplotypes in samples with structural variants. All three tools struggled with diplotypes involving high copy numbers, which, although rare, can be important in certain isolated populations.

Phenotype predictions for Aldy, Astrolabe, and Stargazer generally showed higher concordance than diplotype calls because activity scoring systems often assign correct functional values even when the underlying genotype is miscalled. The study also examined the impact of sequencing coverage and InDel misalignments on accuracy. Limitations included the use of simulated data for rare and structural variants, lack of comparison with custom-targeted panel data, and no assessment of different alignment tools. Novel SNV detection and validation were also not addressed [78]. Both Aldy and Stargazer may generate false-positive or false-negative results for small variants due to reliance on initial read alignments. Another limitation is that two of these tools do not support the GRCh38 genome assembly, requiring investigators to lift alignments to GRCh37.

To overcome these challenges, Chen and colleagues developed Cyrius, a new bioinformatics tool for comprehensive CYP2D6 variant and haplotype calling from WGS data. Cyrius addresses the sequence homology issues between CYP2D6 and CYP2D7 and works with both GRCh37 and GRCh38, achieving a concordance of 99.3% with true genotypes. The tool outperformed Aldy and Stargazer when tested with GeT-RM and long-read datasets, providing improved insights into CYP2D6 diversity across five ethnic groups. The developers plan to expand Cyrius to additional pharmacogenes with paralogs, including CYP2A6 and CYP2B6, and eventually other clinically relevant genes [79].

Understanding the capabilities and limitations of each tool is crucial for their effective application in calling PGx variants from high-throughput sequencing data. Selecting the appropriate algorithm can significantly enhance accuracy and clinical utility.

Overview of computational tools and algorithms for pharmacogenomic analysis from NGS data

Table 3. Key features of the PGx dedicated variant functional prediction tools.

Tool / Algorithm	Main Features and Capabilities	Reference
Stargazer	Calls star alleles from NGS data; detects SNVs, InDels, and structural variants; accurately identifies duplications, deletions, and gene conversions using paralog-specific copy-number analysis	[66]
PharmCAT	Extracts guideline-based variants from VCF files; performs haplotype/diplotype inference; generates interpretable reports with CPIC-based prescribing recommendations	[80]
Aldy	Allelic decomposition of complex, multi-copy pharmacogenes; identifies common, rare, and novel star alleles from whole-genome or targeted sequencing data	[75]
Astrolabe	Fast, automated assignment of CYP2D6 and CYP2D19 activity scores from unphased NGS data; high analytic sensitivity and specificity using a probabilistic scoring system	[81]
Cypripi	Base-pair resolution genotyping of CYP2D6; resolves highly complex structural rearrangements, fusions, duplications, and deletions involving CYP2D6 and pseudogene CYP2D7	[47]
g-Nomic	Clinical PGx interpretation software; provides drug–gene and drug–drug–gene interaction recommendations tailored to individual genotypes and polymedication profiles	[76]
PHARMIP	Integrates structural drug modeling with bioinformatics databases to predict and explain genetic contributions to adverse drug reactions	[77]
Cyrius	State-of-the-art CYP2D6 caller; highest accuracy among current tools; calls all variant types (SNVs, InDels, CNVs, hybrids) and supports both GRCh37 and GRCh38 assemblies	[79]

Strategies for addressing challenges in clinical implementation of NGS-based PGx testing

Several key challenges arise when integrating NGS-based pharmacogenomic testing into routine clinical practice, and strategies to address them are essential.

The first major challenge is the setup and initiation of NGS-based PGx testing in clinical centers. Implementing such testing requires significant investment, including bioinformatics infrastructure, specialized software, computational tools, and trained personnel for data interpretation. Validation studies are also critical to establish the clinical utility and accuracy of the tests. Once these requirements are met and assessments are positively received by public and private payers, NGS-PGx tests can be integrated into standard practice. Costs vary widely depending on the type of test, whether it is a pre-emptive panel or derived from repurposed diagnostic WES/WGS data. Currently, limited insurance coverage remains a major barrier. Increasing physician awareness, expanding third-party support, leveraging direct-to-consumer genetic testing, and reducing costs through technological advancements may help increase clinical uptake and reimbursement [82]. While many services remain reactive single-gene tests, some institutions, such as St. Jude Research Hospital, offer routine pre-emptive PGx testing for all patients (www.stjude.org/pg4kds). A recent U.S. study by Anderson *et al*. found that only a small subset of core pharmacogenes—including CYP2C19, CYP2D6, CYP2C9, VKORC1, UGT1A1, and HLA class I—were typically covered by insurance [83].

A second challenge relates to the limitations of conventional computational prediction tools for pharmacogenes. Many PGx genes are less constrained by evolutionary conservation, reducing the predictive accuracy of standard algorithms. This is particularly true for genes involved in endogenous substance transport, such as OTC1. To address this, combining multiple optimized bioinformatics tools—often six to seven—is recommended for accurate allele imputation of single- or multi-marker PGx signatures. These approaches improve the identification of predictive variants and their likely impact on drug response [67, 84]. Novel variants in evolutionarily conserved positions may still be assessed with standard predictive tools, but in the absence of clinical data, both computational and laboratory validation are required to guide genotype-informed therapy [85, 86].

Other PGx-specific algorithms have been developed to predict loss-of-function or neutral variants with high sensitivity and specificity. These tools provide quantitative estimates of variant effects on gene function. A comprehensive review of computational prediction methods and their application to non-coding variants in drug-metabolizing enzymes and transporters is provided by Zhou *et al*. (2018) [87]. Once functional impact is predicted, the consequences on drug pharmacology can be explored using pathway and molecular interaction databases such as DAVID, Human Metabolome Database, STRING-db, and KEGG. PGx-specific tools including Aldy, Stargazer, Astrolabe, and Cyrius further support NGS data processing and functional interpretation [66, 74]. **(Table 4)** provides an overview of key databases useful for interpreting clinical PGx results.

After establishing a likely functional effect, in-vitro studies or cell line experiments may be employed to confirm the impact of the variant or diplotype on protein activity. However, these approaches are not practical for routine clinical use due to the increased turnaround time. Finally, clinical association studies can validate the relationship between novel variants and patient drug response phenotypes, though this step is suitable only for analyzing individuals with observable clinical outcomes, not for pre-emptive testing of healthy individuals [88].

Major pharmacogenomics databases and resources

Table 4. Useful databases for PGx results interpretation in the clinical practice.

Database / Resource	Main Activities and Key Features	Website / Link	Primary Reference
PharmGKB	Comprehensive, publicly available knowledgebase aggregating, curating, and integrating data on genetic variation and drug response	https://www.pharmgkb.org	[89]
CPIC	International consortium creating and maintaining peer-reviewed, evidence-based, updatable gene/drug clinical practice guidelines	https://cpicpgx.org	[6]
DPWG	Dutch Pharmacogenetics Working Group: develops evidence-based dosing recommendations; integrates guidelines into clinical decision support systems	https://www.pharmgkb.org/page/dpwg	[5]

PharmVar	Central repository for pharmacogene haplotype structure and allelic variation; essential for accurate star-allele nomenclature and test interpretation	https://www.pharmvar.org	[90]
PMKB	Precision Medicine Knowledgebase focused on clinical interpretation of cancer variants; structured, community-editable, pathologist-reviewed	https://pmkb.weill.cornell.edu	[91]
PharmaADME	Industry-led initiative providing a consensus "Core List" of validated ADME genetic markers for clinical trials and regulatory submissions	http://www.pharmaadme.org	–
Flockhart Table	Interactive reference tool listing drug substrates, inhibitors, and inducers of cytochrome P450 isoforms; widely used for drug-interaction teaching	https://drug-interactions.medicine.iu.edu/MainTable.aspx	Flockhart (2007) / updated regularly
SEAPharm	Southeast Asian Pharmacogenomics Research Network; promotes regional collaboration and implementation of pharmacogenomics	–	[92]
PGRN	NIH-funded Pharmacogenomics Research Network (2000–2015); catalyzed major discoveries and translational efforts in pharmacogenomics	https://www.pgrn.org	–
SuperCYP	Comprehensive database and analysis tool for cytochrome P450–drug interactions	https://bioinformatics.charite.de/supercyp/	[93]
FDA Table	Official FDA list of pharmacogenomic biomarkers included in approved drug labeling	https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling	Continuously updated by FDA

While well-characterized and clinically annotated PGx variants can be directly applied in patient care, the integration of newly discovered variants requires substantial supporting evidence. This includes documented gene-drug interactions, phenotyping data, and clinical validation. Typically, these data are initially stored for research purposes, with the possibility of recontacting patients for further investigation. Accurate prediction of an individual's metabolic status is essential for drug dosing decisions, and translating sequencing results into clinically meaningful phenotypes must follow standardized interpretation frameworks. The gene activity score system has been introduced to facilitate this process, converting genotype information into actionable recommendations accepted by reference laboratories and clinical centers [94]. To further enable the clinical adoption of high-throughput PGx reports, healthcare providers need access to evidence-based, practical results supported by standardized cohort and case studies [95, 96].

Conclusion

NGS technologies have been applied in pharmacogenomic research for over a decade. Advances in sequencing platforms, bioinformatics tools, and decreasing costs are now enabling the assessment of larger panels of drug-related genes and biomarkers. Key challenges remain, including the interpretation and management of VUS, the lack of specialized variant-calling software, incomplete haplotype phasing, insufficient coverage of certain genomic regions, and limited experimental or computational means to assess variant functionality. Despite these obstacles, the clinical application of NGS in PGx is steadily expanding, facilitating the discovery of novel variants and supporting the broader implementation of pharmacogenomics-guided therapy. The continued development of high-throughput sequencing methods promises a promising future for personalized medicine and optimized drug treatment.

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