**Review article**

# The Clinical Utility of CYP2D6 Molecular Methods for Personalized Pain Management

**Madisson Hall[1*], Stephanie Jacobson[1] and Patricia Tille[1]**

*Medical Laboratory Sciences Program, College of Allied Health Science, University of Cincinnati, Cincinnati, Ohio, USA[1]*

**As the world faces the persisting opioid epidemic ravaging the globe, one of the ways to combat the overuse and misuse of opioids will be through a personalized approach to pain management. The clinical implementation of pharmacogenetic testing is essential to creating patient-centered drug therapies for the safe and effective treatment of pain. Opioids are used for pain management, but the analgesic affect is different on every individual due to genetic variation and the ability to metabolize the drugs. The highly polymorphic CYP2D6 gene is responsible for the metabolism of the commonly prescribed opioids. Genotype and copy number variation of CYP2D6 play a critical role in how affectively the body metabolizes opioids. A wide variety of molecular methodologies are available for the genotype and copy number analysis of CYP2D6 including real-time polymerase chain reaction, sequencing, microarray, and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy. While these technologies have advanced significantly, there are still limitations and challenges associated with integrating pharmacogenetic testing into routine clinical practice. Further research is needed to establish standardized pharmaceutical recommendation guidelines based on CYP2D6 analysis. However, there is compelling evidence that suggests CYP2D6 testing is useful when considering prescribing opioids for pain management. Implementing pharmacogenetic testing for CYP2D6 may reduce aggressive prescription practices of opioids which, in time, will diminish the devastating effects of the opioid crisis.**

***Keywords:*** Opioid, CYP2D6, pharmacogenetics, pain management

Accepted: May 13, 2023

*Corresponding author: Madisson Hall. E-mail: hall3mp@mail.uc.edu

## Introduction

With recent advancements in genetics, medicine is shifting from a "one size fits all" approach to a customized method based on each individual patient's genetic profile. This emerging practice, called precision medicine, is also known as personalized medicine.[1] One of the ways clinicians can personalize medical treatment is through the use of pharmacogenetic testing. Pharmacogenetic testing allows medical providers to make informed decisions about drug therapies based on how an individual patient will metabolize the medication.

Genetics play a crucial role in how the body metabolizes medications. Variations in metabolism can result in a drug having a wide range of different effects on people. This is particularly important within the context of prescribing medications to control pain management. There are specific genes that play a particularly significant role in the drug metabolism process. Cytochrome P450, also referred to as CYP, represents a large heme-containing enzyme superfamily.[1] CYP enzymes are abundant in the liver and are responsible for the metabolism of various molecules such as drugs, chemicals, and fatty acids.[1] Among the several genes that encode these CYP members is the highly polymorphic CYP2D6 gene located on chromosome 22q13.1.[2] It is estimated that CYP2D6 actively metabolizes around 25 percent of all drugs including antidepressants, antiarrhythmics, antipsychotics, ß-blockers, and opioid analgesics used to treat pain.[1,3]

Due to the variation in CYP2D6, individuals metabolize opioids at different rates. Identification of the genetic variation in the CYP2D6 of each patient by implementing pharmacogenetic testing in routine clinical practice allows providers to take a personalized approach to pain management, leading to safer and more effective treatment. In addition, the clinical practice of genotyping CYP2D6 may reduce the over prescribing of opioids and indirectly assist in the management and reduction of the opioid crisis worldwide.

## Pain Management and the Opioid Crisis

Safe and effective pain therapies require a personalized approach to treatment. Pain is subjective and can be difficult to treat. Individual patients can have a wide range of responses to pain medications based on their genetic profiles. Implementing pharmacogenetic testing in clinical pain management and the prescribing of narcotics is particularly important.

Narcotics or opioids are a class of drugs commonly prescribed for long- and short-term pain management. As a result, opioid addiction has become an increasingly prevalent issue which has led to the opioid epidemic or opioid crisis. In the United States, opioid use has risen by 10 to 14 times in the past two decades.[4] The rate of opioid prescription more than doubled worldwide between 2001 and 2013, largely affecting the United States, Canada, Australia, and the United Kingdom.[5] The increase in opioid prescriptions, misuse and abuse of the drugs has led to the subsequent rise in opioid-related overdoses and deaths. This is attributed to multiple factors including the increased use of prescription opioids by the growing geriatric population as well as efforts to resolve the previous under-treatment of chronic pain.[5] However, evidence suggests that the main contributor to the opioid crisis is the overaggressive prescription practices encouraged by pharmaceutical manufacturers.[5,6,7]

## Opioids

Opioids are a class of medication prescribed for the treatment and management of pain. Opioids create an analgesic effect which simply refers to pain relief.[8] The commonly used opioids include oxycodone, hydrocodone, methadone, morphine, codeine, tramadol, and the synthetic opioid, fentanyl.[9]

Opioids produce analgesic effects by acting on the presynaptic and postsynaptic terminals of the body's neurons by binding to cell membrane receptors. The presynaptic binding blocks calcium channels to prevent the release of neurotransmitters that contribute to nociception or the sensation of pain.[8] The postsynaptic binding of opioids opens

potassium channels, increasing the required action potential to generate nociceptive transmission.[8] The released neurotransmitters stifle the sensation of pain and create a sense of wellbeing. When the effects of the drug wear off, the body can crave the same feeling of pleasure and wellbeing, generating the potential for addiction.[10]

### *Pharmacogenetic Testing*

Clinically integrating pharmacogenetic testing into pain management practices may help to combat the current opioid crisis by reducing the over-prescription and misuse of opioids. Pharmacogenetics investigates how a specific gene influences the body's response to a given drug. Genetic polymorphisms contribute to the high variability in pharmacokinetics and pharmacodynamics which describe how the body processes and responds to various drugs.[11] Pharmacogenetic testing involves assessing an individual's genetic profile to determine how the individual will respond to drug therapy.

## CYP2D6

### *The Role of CYP2D6 in Drug Metabolism*

CYP2D6 is one of the most important pharmacogenes. Cytochrome P450 2D6 is an enzyme encoded by the CYP2D6 gene sequence which is primarily expressed in the liver.[3] CYP enzymes facilitate reactions during phase I metabolism, the process of adding or exposing a polar functional group such as -$NH^2$ or -OH on a lipophilic drug to increase the hydrophilic nature of the molecule.[12] The reaction creates metabolites transforming a prodrug into an active therapeutic form.[12] If the CYP450 system is inhibited in any way it will lead to a decrease in metabolism and an increase in the drug level, whereas if the system is induced, it will cause metabolism to increase and the drug level to decrease.[12]

Significant variability in interindividual CYP2D6 metabolism occurs because the gene is highly polymorphic. More than one hundred CYP2D6 genetic variants have been identified resulting from point mutations, duplications, insertions, and deletions.[1] The genetic variants contribute to the high variability in opioid metabolism by different individuals. Determining one's genotype for CYP2D6 would insure prescribing the correct dose and type of opioid for effective pain management.

### *CYP2D6 Genotypes and Metabolizer Phenotypes*

Determining an individual's CYP2D6 genotype enables healthcare providers to make informed decisions regarding drug choices and doses based on the patient's CYP2D6 metabolizer phenotype. CYP2D6 allelic variants correspond with specific phenotypes categorized as either a poor metabolizer (PM), intermediate metabolizer (IM), ultrarapid metabolizer (UM), or normal metabolizer (NM). NM and IM are the most common phenotypes comprising an estimated 43% to 67% and 10% to 44% of the general population, respectively.[3] NMs provide the baseline for how most individuals are able to process compounds metabolized by CYP2D6 enzymes. IMs may express slightly less CYP2D6 metabolism than NMs but are not considered to be at substantial risk for adverse reactions or failed treatments when prescribed a compound that is metabolized by CYP2D6. In contrast, PMs are typically at higher risk for failed treatments and adverse drug reactions because these individuals exhibit no CYP2D6 enzyme activity and are therefore more likely to experience diminished analgesic effects from opioids due to the inhibited metabolism of the drug.[3,13,14] UMs are often at high risk of adverse reactions when prescribed compounds metabolized by CYP2D6 because these individuals exhibit increased CYP2D6 enzyme activity which can lead to toxic concentrations of the drug even at low doses.[14,15] While PMs and UMs are less common than NMs and IMs, drug choices and doses for these individuals must be carefully considered to minimize risks of dangerous or ineffective treatments.

The CYP2D6 metabolizer phenotype is determined based on the individual's activity score calculated by adding assigned values of each of the alleles.[3] There are five functional allele types for CYP2D6: normal (also known as wild type), decreased function, severely decreased function, no function, and increased

function. Normal function alleles include **1, *2,* and **35* which are all assigned an activity score of 1.[3,15] Decreased function alleles include **9, *17, *29,* and **41* and are assigned a value of 0.5.[3] The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines also group *CYP2D6*10* in with the decreased function allele type. It is the only decreased function allele with an activity score of 0.25 rather than 0.5.[15] Due to this distinction, the allele can be categorized separately from the rest of the decreased function alleles to highlight the difference in activity values. CYP2D6 alleles that are considered to have no function are given a score of 0 and these include **3, *4, *5, *6,* and **40*.[3,16] Increased function alleles, which are **1x2* and **2x2,* are assigned twice the value of a normal allele, contributing a value of 2 to the activity score.[3] An activity score of 0 indicates a PM phenotype, 0.25 to 1 indicates an IM, 1.25 to 2.25 indicates a NM, and activity scores greater than 2.25 indicate a UM phenotype.[3,15] For example, an individual with a *CYP2D6*1/*2* diplotype has an activity score or 2 making them a NM, whereas an individual with a *CYP2D6*4/*5* diplotype has an activity score of 0 making them a PM.

The frequencies of the alleles and phenotypes vary by population and ethnic groups. No function *CYP2D6*3, *4, *5,* and **6* alleles are common among individuals of European descent.[3] Compared to other biogeographical groups, Europeans exhibit the highest frequency of poor metabolizers.[16] In contrast, Sub-Saharan African populations have the highest prevalence of activity scores of 3.0 and higher.[16] The severely decreased function *CYP2D6*10* allele is common among Asian populations. East Asian populations have the highest rate of 0.25 activity scores relative to other biogeographical groups.[3,16] The frequency of the alleles in a given population affects the chances of an individual in that population being a PM or UM for CYP2D6.

### *CYP2D6 Copy Number Variation*

In addition to genotype, copy number variation (CNV) in CYP2D6 resulting from gene duplications or deletions can also influence metabolism. Two copies of an allele are normally inherited, one from the mother and one from the father.[17] In cases where CNV occurs, two or more copies of a gene may be inherited from one of the parents, or the gene may be deleted altogether.[17] When there is a duplication of the CYP2D6 gene, the additional copy is also factored into the activity score. Therefore, the more copies of CYP2D6 present, the more likely that individual will have increased CYP2D6 function.[3,17] The increased CYP2D6 function causes the individual to metabolize compounds much faster than a person with only one copy of the gene.[17] In contrast, reduced CYP2D6 function resulting from a deletion will inhibit the individual's ability to metabolize the compound, which will reduce or eliminate the intended analgesic effect of the opioid.

Over 12 percent of the United States population has a CNV in the CYP2D6 gene.[17] The total copy number of CYP2D6 can range from zero copies to as high as ten copies.[17] This additional factor of genetic variation can significantly influence the expected metabolism type hindering the process of translating data into patient-specific outcomes. Scientists have previously overlooked the impact of CNV on drug response.[18] Additionally, some researchers have criticized the under-utilization of CYP2D6 CNV testing, particularly in laboratory-developed tests, stating that many of these tests only identify the single nucleotide polymorphisms and do not account for CYP2D6 CNVs.[18] The prevalence of CYP2D6 CNVs combined with the significant contribution to metabolizer phenotypes suggests that CYP2D6 CNV testing should be used alongside genotype analysis for the most accurate and clinically useful results in pharmacogenetic testing.[18]

## CYP2D6 Molecular Methods

There are a variety of molecular test methods used for CYP2D6 testing. The methods include real-time polymerase chain reaction (PCR), sequencing, microarrays, and matrix-assisted

laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Each of the methods come with benefits and limitations when implementing them in a clinical setting.

***Real-time PCR***

PCR is a commonly used method for CYP2D6 genotype analysis in clinical laboratories. There are a variety of PCR tests available for genotyping CYP2D6, however, only a couple are Food and Drug Administration (FDA) approved.[19] The quality and usefulness of CYP2D6 PCR assays depend heavily on the design of the PCR primers specific to the intended target.[20]

A wide variety of PCR chemistries use a direct detection method for genotyping CYP2D6, one being TaqMan chemistry. TaqMan chemistry utilizes two oligonucleotide probes with a fluorescent molecule attached at the 5' end and a quencher located on the 3' end. In TaqMan assays designed for the detection of SNPs, one allele is detected by the fluorescein adamites (FAM) probe and the other by the aequorea victoria (VIC) probe.[21,22] The primers, probes, and master mix containing DNA polymerase, deoxynucleotide triphosphates (dNTPs), cofactor, and buffer are combined with the sample.[23] Then a thermal cycling protocol is performed on a PCR detection instrument such as the Applied Biosystems 7500 where the DNA is amplified and the fluorescence is measured.[24] The endpoint read of the fluorescence is used for genotype analysis.[24] Software programs such as *TaqMan Genotyper Software* analyze the amplification to generate allele plots in which distinct clusters should form.[24] One axis of the allele plot represents the allele detected by FAM and the other axis represents the allele detected by VIC. Clusters that are formed along the FAM axis represent the samples that are homozygous for the FAM-detected allele and those along the VIC axis are homozygous for the VIC-detected allele. A cluster that forms in the middle of the plot equidistant from both axes marks the samples that are heterozygous, meaning both alleles were detected in the samples.[24] TaqMan PCR is a fast and reliable method for CYP2D6 genotyping that is simple to perform compared to other methods.[20]

In addition to genotyping, PCR tests are also available for CNV identification for CYP2D6. The assays typically use TaqMan chemistry with real-time quantitative PCR methods.[19] In TaqMan CNV assays for CYP2D6, one probe labeled with FAM targets the CYP2D6 sequence while another probe labeled with VIC targets a reference gene with a known copy number, such as Ribonuclease P.[24] Both sequences are amplified in the same well simultaneously, and at the end of the PCR, a cycle threshold (Ct) value is determined for both targets. The Ct value is the number of PCR cycles it takes for the target to cross the threshold where it eventually exceeds background amplification.[25]

To determine the number of copies of CYP2D6 in a sample, the difference between the FAM and VIC Ct values are measured for the tested sample and compared to the difference in Ct values measured in a calibrator sample with a known copy number for CYP2D6. Special software automatically performs the calculations and determines a predicted copy number using a data file exported from the PCR instrument. An example of the technology is the *CopyCaller 2.1* software created by Thermo Fisher Scientific (Waltham, MA).[24] The software also provides statistics to assess the confidence of each copy number call and assigns each sample a score. One of the limitations of the technology is that it requires a minimum of seven samples with the same predicted copy number in a single PCR run in order to accurately generate confidence scores for each sample's predicted copy number.[24]

***Sequencing***

Various sequencing techniques are available for CYP2D6 analysis. In fact, the use of Sanger sequencing actually led to the discovery of the CYP2D6 gene and pseudogenes.[2] Eventually, long-range PCR enabled Sanger sequencing of targeted exons across full-length CYP2D6 amplicons, leading to the identification of the

initial CYP2D6 star alleles.[2] As innovative technologies emerge, Sanger sequencing remains the gold-standard for many molecular methods including genotyping applications. However, it is rapidly being replaced in clinical laboratories with higher throughput methods such as targeted next generation sequencing (NGS).[2]

Targeted NGS includes a step where specific gene regions are selectively amplified through PCR using a gene panel. A gene panel is a pool of oligonucleotide primer pairs used to amplify the target region during PCR, creating a sequencing-ready library of DNA amplicons.[26] The DNA is denatured and added to a small plate, sometimes referred to as a flow cell containing oligonucleotides that match the adapter sequences of the library. The adapter sequences on the DNA fragments hybridize with the targets on the plate. PCR synthesizes a complementary DNA (cDNA) sequence. Fragments that fail to hybridize to a complementary sequence are removed in a subsequent washing. The free end of the cDNA sequence then hybridizes to a secondary oligonucleotide on the plate in a process known as bridge building.[27] The bridge is amplified and denatured again, and the process is repeated, creating multiple copies of forward and reverse strands. A primer then binds to the oligonucleotide to start the sequencing process using fluorescently labeled nucleotides. As the nucleotides bind to the sequence, they are excited by a laser to obtain a color-coded signal until the sequencing is complete, generating millions of reads in the process.[27]

While NGS offers a rapid and reliable method for detecting SNPs in CYP2D6, the massive amount of data produced by the millions of reads requires extraordinarily complex bioinformatic systems to analyze the results. Another limitation of this method for CYP2D6 testing is the challenge associated with detection of CNVs which typically requires the use of quantitative real-time PCR.[2,28] The challenges are caused by the variances in coverage depth often associated with NGS methods. The variances are due to the amount of GC content in the target regions and biochemical properties of the kits used in the initial enrichment steps prior to sequencing.[28]

### *Microarrays*

A substantial portion of all pharmacogenomic tests use microarray method. Microarrays use a grid that contains small wells, each of which contains multiple copies of a probe fixed to a solid surface in the well. Each well represents a different gene or region of interest. The sample DNA is denatured and cut into smaller, more manageable fragments.[29] The small fragments are labeled by attaching a fluorescent dye. The labeled sample DNA is inserted into the wells where it hybridizes with complementary probes.[29] Any unbound DNA is then washed away, and the bound DNA fluoresces resulting in the identification of a specific gene arrangement or variation.

Microarrays are commonly used for pharmacogenomic applications because the technology can analyze thousands of genetic variants simultaneously, including CNV.[30] Other advantages of microarray technology include rapid output, affordability, and availability of the technology as well as high accuracy and relatively simple analysis and variant calling compared to other methods.[31 - 33] Microarray panels can be customized to include specific genes of interest to create pharmacogenomic panels to identify the variants important in pain management, cardiac, and psych disorders.[31,33] The main limitation associated with microarray technology is that it cannot detect novel variants. However, genome-wide array technology can detect virtually all SNPs of known clinical importance making it suitable for clinical applications.[31] In fact, multiple assays that detect drug metabolizing enzymes that have been granted FDA approval utilize microarray technology.[19]

### *MALDI-TOF Mass Spectrometry*

One of the less commonly used methodologies for CYP2D6 genotyping is MALDI-TOF MS. In this method, forward and reverse primers are created for the target SNP and a PCR step is

performed to amplify the region of DNA containing the SNPs.[34,35] An extension step is then performed during which an extension primer anneals to the polymorphic base and a terminator extends the fragment by a single additional base.[34] The product of the reaction is added to a chip containing matrix solution. The matrix assists in the ionization process by absorbing energy from a laser.[34,35] Electrostatic potential accelerates the ionized DNA molecules through a tube toward a detector which measures the relative time of flight of each molecule.[34] The mass of the DNA fragment is calculated. The modified terminator bases enable detection of mass differences between fragments differing by only one base.[34] This method is used to detect SNPs based on the mass of the variant sequence.[34 - 36]

Tests using MALDI-TOF MS are proving to be a competitive analytical method due to the many benefits including rapid high throughput, ability to customize, relatively easy setup protocols, and low cost per test.[35,36] Additionally, MALDI-TOF MS technology has demonstrated high accuracy, sensitivity, and specificity for pharmacogenetic testing.[35,36] There is currently only one MALDI-TOF MS-based genotyping assay approved for clinical use in the United States, however it is not for CYP2D6 genotyping.[19]

## Discussion

Pharmacogenetic testing for CYP2D6 is essential to determine a patient's ability to metabolize a compound appropriately and predict the response to CYP2D6-metabolized drugs such as opioids. Both genotype and copy number variation analyses provide critical information that can help health care providers prescribe appropriate treatments and dosages based on the genetic profile of the patient.

Understanding the clinical utility of CYP2D6 testing for personalized pain management is more important than ever given the current opioid crisis. Opioid use has increased by more than 10 times in the last 20 years, and it has been predicted that 480,000 people in the United States could die from opioid overdose in the next 10 years.[5,37] The recent COVID-19 pandemic may have contributed to the severity of the worldwide opioid problem due to intense social isolation, increased experiences of grief and trauma, and limited access to an already low number of in-person treatment centers for people struggling with opioid abuse.[37]

Also contributing to the high use of opioids is the prevalence of opioids like oxycodone as a postoperative treatment for pain. Of the 3.9 million surgeries undergone by children each year in the United States, oxycodone is prescribed for 2,116 out of every 100,000 patients.[38] Despite the high variability in response to the medication and the increased risk for opioid dependence, oxycodone is the most commonly prescribed oral opioid for children.[38]

The immense threat that the overuse and misuse of opioids poses to the population highlights the urgent need for an increased awareness and understanding of the utilization of CYP2D6 testing to aid in prescribing safe and effective treatment for pain. Evidence suggests that in addition to oxycodone dosage requirements, CYP2D6 genotypes may also affect a patient's risk for opioid side-effects like respiratory depression.[38] This contradicts current information provided by the CPIC which stated that "there is insufficient evidence and confidence to provide a recommendation to guide clinical practice at this time for oxycodone" based on CYP2D6 genotype.[15]

While the current research available may be "insufficient" at this time, there is enough substantial evidence to warrant further investigation and consideration for clinical implementation. Studies have shown that genotype availability and guidance in a clinical setting influences postoperative prescriptions of opioids. These effects include a decrease in the prescription of a medication due to high variability in response to the drug as well as a

lower rate of oxycodone prescriptions for PMs and UMs.[38,39]

One of the major challenges of integrating CYP2D6 testing into routine clinical practice is the lack of standardization of activity scores, associated phenotypes, and the clinical recommendations for each phenotype. Translating CYP2D6 genotype to phenotype has posed a significant challenge to the scientific community.[40] CPIC guidelines on converting CYP2D6 activity scores to metabolizer phenotype have been modified in recent years, primarily changing the activity score ranges that constitute an IM, NM, and UM.[41,42] A recent study found that the metabolic ratio (the ratio of parent drug to metabolite after 3 hours) of three different CYP2D6 genotype groups was significantly different, even though all three groups would be classified as NMs according to CPIC guidelines.[40] The clinical implications of these differences need to be carefully considered when establishing or modifying the activity score translation system. To produce sufficient comparable data to aid in establishing guidelines for prescription recommendations for each phenotype, it would be beneficial to adopt one standard CYP2D6 genotype-to-phenotype translation system.

One of the factors that is improving the ability to implement pharmacogenetic testing in clinical laboratories is that the same molecular methodologies used for CYP2D6 can also be used for a variety of other important pharmacogenes, including other members of the Cytochrome P450 superfamily such as CYP2C9 and CYP2C19.[43] Once a molecular method for a gene such as CYP2D6 has been implemented, it can be a relatively straightforward process to continue implementing additional assays to monitor other genes, as most principles and procedures are transferable to other pharmacogenes, apart from the unique primer designs.

There are a variety of molecular methodologies currently available for pharmacogenetic testing including PCR, sequencing, microarray, and MALDI-TOF MS. While molecular methods for genetic testing have improved, leading to more sensitive and less labor-intensive tests, there are still limitations associated with these methods that remain a challenge for clinical laboratories. One of the current limitations of copy number analysis is that most CNV assays do not identify which alleles have multiple copies.[15] The main challenge associated with genotype analysis across all molecular methods is the risk of incorrect variant calling.[44] For instance, rare and de novo variants will not be detected by most assays and may be falsely reported as a functional allele by default.[15] Regardless of the diagnostic tool being used, laboratorians should be especially cautious when analyzing variants in homologous regions to reduce the chance of an incorrect call.[44] In some cases, incorrect variant calls can in turn be translated into incorrect phenotypes, which may have significant negative outcomes for the patient. Ambiguous results and variants in highly homologous regions require confirmation with an independent methodology to help avoid this risk.

## Conclusion

In order to continue developing and refining strategies for personalized medical treatment it is necessary to assess the clinical utility and challenges associated with current practices. Pharmacogenetic testing is a valuable tool in personalized medicine due to the influence that genetic variation has on the diversity of drug metabolism within and between populations. In addition to pain management, pharmacogenetic testing has other useful clinical applications. Gene sequences are grouped to create different test panels including cardiovascular, psychiatric, cancer and immunosuppression, and endocrine and metabolic panels.

CYP2D6 testing has proven to be a valuable way to predict an individual's response to opioids as well as other CYP2D6-metabolized drugs. Aside from pain medications, CYP2D6 testing may be clinically useful for the

prescription of antidepressants, antiarrhythmics, antipsychotics, and ß-blockers. In the new era of personalized medicine, CYP2D6 testing will play a pivotal role in determining an individual's best drug treatment options.

## References


1. Ahmed S, Zhou Z, Zhou J, Chen SQ. Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine [published correction appears in Genomics Proteomics Bioinformatics. 2018 Apr 21]. Genomics Proteomics Bioinformatics. 2016;14(5):298-313. doi:10.1016/j.gpb.2016.03.008
2. Yang Y, Botton MR, Scott ER, Scott SA. Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing. Pharmacogenomics. 2017;18(7):673-685. doi:10.2217/pgs-2017-0033
3. Kane M. CYP2D6 overview: allele and phenotype frequencies. Med Genet Sum [Internet]. 2021 Oct [cited 2023 Apr 10]. Available from: https://www.ncbi.nlm.nih.gov /books/NBK574601/.
4. U.S. Food & Drug Administration [Internet]. Silver Spring: U.S. Food and Drug Administration; c2018 [cited 2023 Apr 10]. Precision Medicine; Available from: https://www.fda.gov/medical-devices/in-vitro-diagnostics/precision-medicine#:~:text=Precision%20medicine%2C%20sometimes%20known%20as,genes%2C%20environments%2C%20and%20lifestyles.
5. Shipton EA, Shipton EE, Shipton AJ. A Review of the Opioid Epidemic: What Do We Do About It?. Pain Ther. 2018;7(1):23-36. doi:10.1007/s40122-018-0096-7
6. Robert M, Jouanjus E, Khouri C, Fouilhé Sam-Laï N, Revol B. The opioid epidemic: A worldwide exploratory study using the WHO pharmacovigilance database. Addiction. 2023 Apr;118(4):771-775. doi: 10.1111/add.16081. Epub 2022 Nov 22. PMID: 36331523.
7. Celentano D. The Worldwide Opioid Pandemic: Epidemiologic Perspectives. Epidemiol Rev. 2020;42(1):1-3. doi:10.1093/epirev/mxaa012
8. Cohen B, Ruth LJ, Preuss CV. Opioid Analgesics. In: StatPearls. Treasure Island (FL): StatPearls Publishing; October 10, 2022.
9. Murphy DL, Lebin JA, Severtson SG, Olsen HA, Dasgupta N, Dart RC. Comparative Rates of Mortality and Serious Adverse Effects Among Commonly Prescribed Opioid Analgesics. Drug Saf. 2018;41(8):787-795. doi:10.1007/s40264-018-0660-4.
10. Mayo Clinic Staff, editors. How opioid addiction occurs [Internet]. Rochester: Mayo Foundation for Medical Education and Research; 2022 [cited 2023 Apr 10]. Available from: https://www.mayoclinic.org/diseases-conditions/prescription-drug-abuse/in-depth/how-opioid-addiction-occurs/art-20360372.
11. Bishop JR. Pharmacogenetics. Handb Clin Neurol [Internet]. 2018 Jan [cited 2023 Apr 10]; 147(1). Available from: https://doi.org/10.1016/B978-0-444-63233-3.00006-3.
12. Ahmed S, Zhou Z, Zhou J, Chen SQ. Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine [published correction appears in Genomics Proteomics Bioinformatics. 2018 Apr 21]. Genomics Proteomics Bioinformatics. 2016;14(5):298-313. doi:10.1016/j.gpb.2016.03.008
13. Yang Y, Botton MR, Scott ER, Scott SA. Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing. Pharmacogenomics. 2017;18(7):673-685. doi:10.2217/pgs-2017-0033
14. Kane M. CYP2D6 overview: allele and phenotype frequencies. Med Genet Sum [Internet]. 2021 Oct [cited 2023 Apr 10]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK574601/.

15. Phang-Lyn S, Llerena VA. Biochemistry, Biotransformation. In: StatPearls. Treasure Island (FL): StatPearls Publishing; August 22, 2022.
16. T Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD, Rawson R. Trends in Tramadol: Pharmacology, Metabolism, and Misuse. Anesth Analg. 2017;124(1):44-51. doi:10.1213/ANE.0000000000001683.
17. Zhu Y, Lopes GS, Bielinski SJ, et al. Impact of Pharmacogenomic Information on Values of Care and Quality of Life Associated with Codeine and Tramadol-Related Adverse Drug Events. Mayo Clin Proc Innov Qual Outcomes. 2021;5(1):35-45. Published 2021 Jan 13. doi:10.1016/j.mayocpiqo.2020.08.009.
18. Crews KR, Monte AA, Huddart R, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6, OPRM1, and COMT Genotypes and Select Opioid Therapy. Clin Pharmacol Ther. 2021;110(4):888-896. doi:10.1002/cpt.2149.
19. PharmGKB [Internet]. Stanford: PharmGKB; c2001-2023 [cited 2023 Apr 10]. Gene-specific Information Tables for CYP2D6. Available from: https://www.pharmgkb.org/page/cyp2d6RefMaterials.
20. Jarvis JP, Peter AP, Shaman JA. Consequences of CYP2D6 Copy-Number Variation for Pharmacogenomics in Psychiatry. Front Psychiatry. 2019;10:432. Published 2019 Jun 20. doi:10.3389/fpsyt.2019.00432
21. Beoris M, Amos Wilson J, Garces JA, Lukowiak AA. CYP2D6 copy number distribution in the US population. Pharmacogenet Genomics. 2016;26(2):96-99. doi:10.1097/FPC.0000000000000188.
22. U.S. Food & Drug Administration [Internet]. Silver Spring: U.S. Food and Drug Administration; c2023 [cited 2023 Apr 10]. Nucleic Acid Based Tests. Available from: https://www.fda.gov/medical-devices/in-vitro-diagnostics/nucleic-acid-based-tests.
23. Larsen JB, Rasmussen JB. Pharmacogenetic testing revisited: 5' nuclease real-time polymerase chain reaction test panels for genotyping CYP2D6 and CYP2C19. Pharmgenomics Pers Med. 2017;10:115-128. Published 2017 Apr 18. doi:10.2147/PGPM.S131580.
24. Ye H, Lu Q, Duan N, Wang Z. GO-amplified fluorescence polarization assay for high-sensitivity detection of aflatoxin B1 with low dosage aptamer probe. Anal Bioanal Chem. 2019;411(5):1107-1115. doi:10.1007/s00216-018-1540-6.
25. Lambert GG, Depernet H, Gotthard G, et al. Aequorea's secrets revealed: New fluorescent proteins with unique properties for bioimaging and biosensing. PLoS Biol. 2020;18(11):e3000936. Published 2020 Nov 2. doi:10.1371/journal.pbio.3000936.
26. MilliporeSigma [Internet]. Darmstadt: MilliporeSigma; c2023 [cited 2023 Apr 10]. PCR Master Mix. Available from: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/genomics/pcr/pcr-master-mix.
27. ThermoFisher Scientific, editor. Pharmacogenomics Experiments Application Guide [Internet]. Waltham: ThermoFisher Scientific; 2022 [cited 2023 Apr 10]. Avalable from: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0009612_Pharamacogenomics_UG.pdf&title=QXBwbGljYXRpb24gR3VpZGU6IFBoYXJtYWNvZ2Vub21pY3MgRXhwZXJpbWVudHM=.
28. ThermoFisher Scientific, editor. Data Analysis on the ABI PRISM® 7700 Sequence Detection System: Setting Baselines and Thresholds [Internet]. Waltham: ThermoFisher Scientific; 2022 [cited 2023 Apr 10]. Available from: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FApplication-Notes%2Fcms_042502.pdf&title=QXBwbGljYXRpb24gTm90ZXMgJmFtcDsgVHV0b3JpYWxzOiBEYXRhIEFuYWx5c2lzIG9uIHRoZSBBQkkgUFJJU00mcmVnOyA3NzAwIFNlcXVlbmNlIERldGVjdGlvbiBTeXN0ZW06IFNldHRpbmcgQmFz

ZWxpbmVzIGFuZCBUaHJlc2hvbGRzOiBHdWlkZTogUmV2IEE=.
29. Kim MJ, editor. Complete Guide to Ion Ampliseq Technology – How Does Ion Ampliseq Technology Work? [Internet]. Waltham: ThermoFisher Scientific; 2017 [cited 2023 Apr 10]. Available from: https://www.thermofisher.com/blog/behindthebench/complete-guide-to-ion-ampliseq-technology-how-does-ion-ampliseq-technology-work/.
30. Illumina, Inc. An Introduction to Next-Generation Sequencing Technology [Internet]. San Diego: Illumina, Inc.; 2017 [cited 2023 Apr 10]. Available from: https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf.
31. May V, Koch L, Fischer-Zirnsak B, et al. ClearCNV: CNV calling from NGS panel data in the presence of ambiguity and noise. Bioinformatics. 2022;38(16):3871-3876. doi:10.1093/bioinformatics/btac418.
32. National Human Genome Research Institute [Internet]. Bethesda: National Institutes of Health; c2020 [cited 2023 Apr 10]. DNA Microarray Technology Fact Sheet. Available from: https://www.genome.gov/about-genomics/fact-sheets/DNA-Microarray-Technology.
33. Mou X, Ali Z, Li B, Li T, Yi H, Dong H, He N, Deng Y, Zeng X. Multiple genotyping based on multiplex PCR and microarray. Chin Chem Let [Internet]. 2016;27(11):1661-1665. Published 2016 Nov. Available from: https://www.sciencedirect.com/science/article/abs/pii/S100184171630078X.
34. Shahandeh A, Johnstone DM, Atkins JR, et al. Advantages of Array-Based Technologies for Pre-Emptive Pharmacogenomics Testing. Microarrays (Basel). 2016;5(2):12. Published 2016 May 28. doi:10.3390/microarrays5020012.
35. Krebs K, Milani L. Translating pharmacogenomics into clinical decisions: do not let the perfect be the enemy of the good. Hum Genomics. 2019;13(1):39. Published 2019 Aug 27. doi:10.1186/s40246-019-0229-z.
36. Medwid S, Kim RB. Implementation of pharmacogenomics: Where are we now? [published online ahead of print, 2022 Nov 10]. Br J Clin Pharmacol. 2022;10.1111/bcp.15591. doi:10.1111/bcp.15591.
37. Ellis JA, Ong B. The MassARRAY® System for Targeted SNP Genotyping. Methods Mol Biol. 2017;1492:77-94. doi:10.1007/978-1-4939-6442-0_5.
38. Li D, Yi J, Han G, Qiao L. MALDI-TOF Mass Spectrometry in Clinical Analysis and Research. J Am Chem Soc [Internet]. 2022 Aug [cited 2023 Apr 10];2(5). Available from: https://pubs.acs.org/doi/full/10.1021/acsmeasuresciau.2c00019.
39. Cui JJ, Wang LY, Tan ZR, Zhou HH, Zhan X, Yin JY. MASS SPECTROMETRY-BASED PERSONALIZED DRUG THERAPY. Mass Spectrom Rev. 2020;39(5-6):523-552. doi:10.1002/mas.21620.
40. Hornberger J, Chhatwal J. Opioid Misuse: A Global Crisis. Value Health. 2021;24(2):145-146. doi:10.1016/j.jval.2020.12.003.
41. Cavallari LH, Cicali E, Wiisanen K, et al. Implementing a pragmatic clinical trial to tailor opioids for acute pain on behalf of the IGNITE ADOPT PGx investigators. Clin Transl Sci. 2022;15(10):2479-2492. doi:10.1111/cts.13376.
42. Merchant S, Prows CA, Yang F, et al. Association of CYP2D6 genotype predicted phenotypes with oxycodone requirements and side effects in children undergoing surgery. Ann Transl Med. 2022;10(23):1262. doi:10.21037/atm-2022-58.
43. Jin Y, Zhang S, Hu P, et al. The impact of CYP2D6*41 on CYP2D6 enzyme activity using phenotyping methods in urine, plasma, and saliva. Front Pharmacol. 2022;13:940510. Published 2022 Aug 30. doi:10.3389/fphar.2022.940510.
44. Hicks JK, Sangkuhl K, Swen JJ, et al. Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. Clin Pharmacol

Ther. 2017;102(1):37-44. doi:10.1002/cpt.597.
45. Caudle KE, Sangkuhl K, Whirl-Carrillo M, et al. Standardizing CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. Clin Transl Sci. 2020;13(1):116-124. doi:10.1111/cts.12692.
46. Gaedigk A, Ingelman-Sundberg M, Miller NA, et al. The Pharmacogene Variation (PharmVar) Consortium: Incorporation of the Human Cytochrome P450 (CYP) Allele Nomenclature Database. Clin Pharmacol Ther. 2018;103(3):399-401. doi:10.1002/cpt.910.
47. Nfonsam L, Ordorica S, Ghani M, et al. Leveraging the power of new molecular technologies in the clinical setting requires unprecedented awareness of limitations and drawbacks: experience of one diagnostic laboratory. J Med Genet. 2019;56(6):408-412. doi:10.1136/jmedgenet-2018-105443.