P23 LABS

TRUSTED LABORATORY EXCELLENCE

PGX TESTING BY P23 LABS

SCIENTIFIC EVIDENCE WHITE PAPER TO SUPPORT CLINICAL USE OF PHARMOGENOMICS IN MEDICATION MANAGEMENT AND USE IN PATIENTS

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An Overview of the PGx Testing as the best solution for interindividual drug responses and medication usage, prescription, and patient management.

EXECUTIVE OVERVIEW

The future of **personalized medicine** and **consumer-driven medicine** holds endless possibilities. We, at P23 Labs, can simplify testing, improve the efficiency of molecular diagnostics, reduce consumer and corporate costs, increase overall access to diagnostics, and increase the quality of patient care domestically and internationally. P23 Labs is poised to take full advantage of the opportunities available on the market to pursue its mission to diagnose and detect disease at a time when it is most clearly needed while providing insight into individualized treatment.

P23 Labs is a clinical reference laboratory that provides preventative diagnostics testing and molecular diagnostics services. We are dedicated to promoting the widespread use of molecular genetic screens to detect early signs of DNA and cellular damage, while continually advancing/expanding repertoire of complementary diagnostic services. Our current line consists of Wellness and Pharmacogenetics (PGx) Testing. Our molecular genetic screenings are easy to administer and include non-invasive options. These diagnostic screens are developed to diagnose cancer, infectious diseases, and inherited conditions.

At P23 Labs, we are driven to provide innovative, high-quality, and affordable customer-focused diagnostic testing. We seek out partnerships based on clear pricing and top-tier service. Client Services is actively establishing partnerships with public health departments and community-based organizations that serve individuals suffering from a variety of health disparities.

Today, P23 Labs encompasses an incredibly driven team of passionate salespeople and specialized doctors who work to merge the gap between diagnostics and results. Doing business with P23 Labs guarantees a high-quality Customer Experience and direct access to lab directors. As the global genetic diagnostics industry expands, the need for more personalized medical solutions follows suit. P23 is well equipped to meet these rising demands—our laboratory boasts an extensive line of screenings, preventative diagnostics, and unique testing algorithms. We aim to provide these exceptional services to our clients by adhering to the following principles of success:

- ✓ Value-based and client-centered service
- ✓ Regulation and Federal Compliance
- ✓ Partnerships with proactive as opposed to reactive healthcare establishments (where care is integrated into daily practice processes)

- ✓ Company culture that revolves around collaboration, scientific innovation, and investment in state-of-the-art technology
- ✓ Reliable, high-precision screenings/risk assessments
- ✓ Revenue diversification
- ✓ Ensure pricing policy that is appropriate
- ✓ Actionable screen results and services
- ✓ Cost structure of tests and reimbursement procedures supported by target markets
- ✓ Maximum utility in laboratory (economies of scale)
- ✓ Team of intelligent, passionate, and ethical employees
- ✓ Innovative research and development
- ✓ Utilize health information technology (HIT) and social media platforms to encourage positive physician/patient experience

INTRODUCTION

Pharmacogenetics is focused on understanding how individuals differ in their response to medications based on their genetic profiles. Several factors, such as gender, age, and diet, influence one's response to medications; genetics are no exception. Genes responsible for encoding the metabolic pathways on which drugs act can impact the therapeutic and sometimes adverse effects of drug molecules.

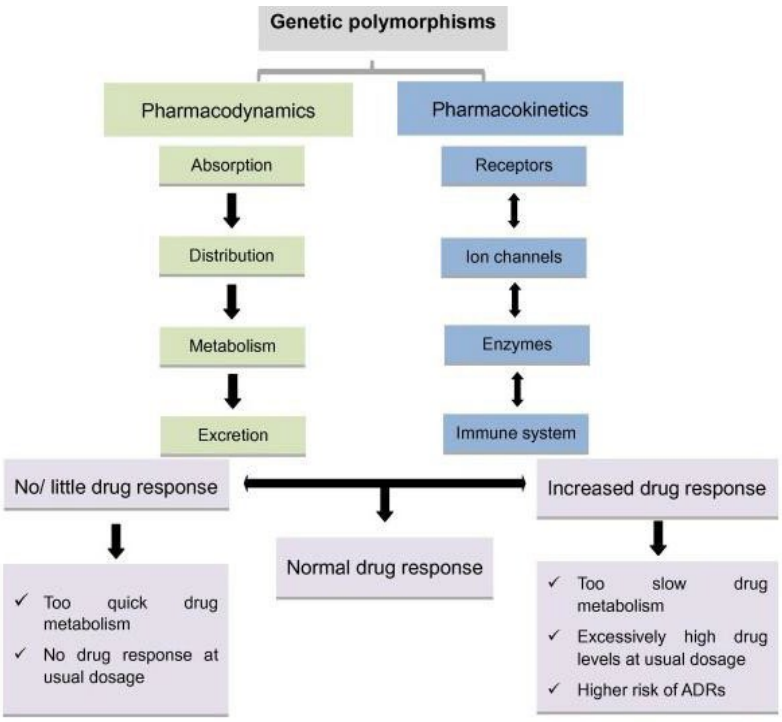


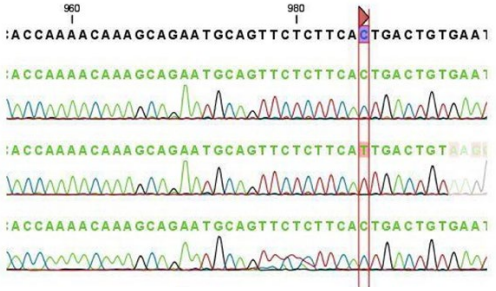
Figure 1. Effect of genetic polymorphisms on individuals' drug response

*"**Pharmacokinetics** and **pharmacodynamics** are primary determinants of interindividual differences in drug responses. **Genetic polymorphism** in genes related to these processes may result in mild to severe variations in drug responses or ADRs (adverse drug reactions). (Ahmed, S., Zhou, Z., Zhou, J., & Chen, S. (2016))*

In addition, pharmacogenetics also has the potential to lessen the number of adverse drug reactions. ADRs are the fourth leading cause of death in the United States, with 2.2 million cases annually. Hence, pharmacogenetic analysis and other environmental play an important role in determining genomic differences. With over 14 million Single Nucleotide Polymorphisms (SNPs) distributed throughout the human genome, differences occur approximately every 300–1000 nucleotides.

Genetic Testing | Single Nucleotide Polymorphisms (SNPs)

- 99.9% of DNA sequencing is identical in humans.
- One of the most common forms of genetic variations (in the .1%) in humans is called the **single nucleotide polymorphism**
- **SNPs** are single nucleotide changes that vary from person to person.
- SNPs occur about every 100–300 base pairs, and most of them are in non-coding regions of DNA.
- If an SNP occurs in a gene sequence, it can produce disease or confer susceptibility to a disease.



Additionally, variation in genetic information due to SNPs linked to drug metabolizing enzymes has been shown to greatly affect drug absorption, metabolism, and excretion of these compounds. Therefore, the identification of DNA variants that significantly contribute to population variations in each trait is one of the fundamental objectives of genetics (Ahmed, 2016). Recent pharmacogenomics have greatly improved our understanding of variations in drug response behavior. Genetic variations in patient populations can help (not clear on what is being said here) a treatment proven to be efficacious in some patients can fail to elicit adequate responses in others. Moreover, treatment failure in affected patients can cause serious side effects or death.

The complex, multifold causative factors for variations in drug response carry many direct and indirect consequences. Inherited genetic factors have the most influence, but other variables include environmental factors like chemicals and radiation exposure, lifestyle factors like drinking, smoking, and exercise, and physiological factors like age, sex, liver and kidney function, pregnancy, and starvation. It is evident from previous studies that population variability in drug response is often larger than interpatient variability (within the same individual at different time points) (Ahmed, 2016).

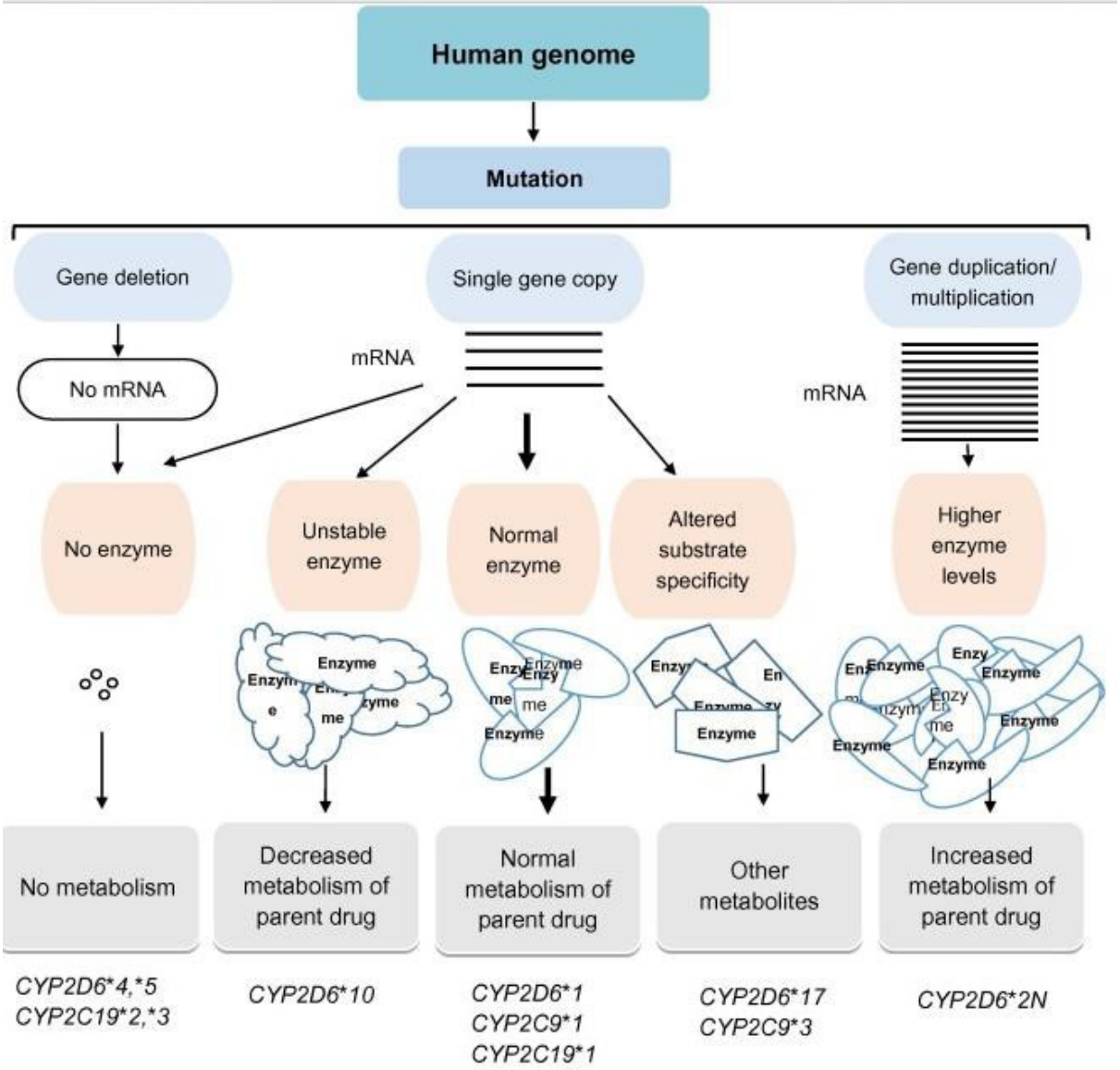


Figure 2: An overview of important consequences of genetic polymorphisms in CYPs.

Overview of the effect of genetic polymorphisms on some human cytochrome P450 variant alleles and molecular mechanisms leading to altered drug metabolism.

Drug response of individual patients is primarily determined by the pharmacokinetic and pharmacodynamic properties of prescribed drugs. These properties can be directly or indirectly affected by polymorphisms in drug metabolizing enzymes and transporters. Different populations contain varied allele frequencies in genes that encode drug-metabolizing enzymes and transporters.

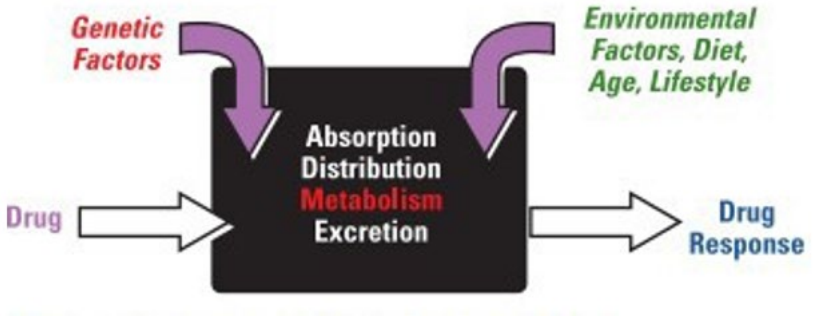


Figure 4: Factors responsible for variations in drug responses.

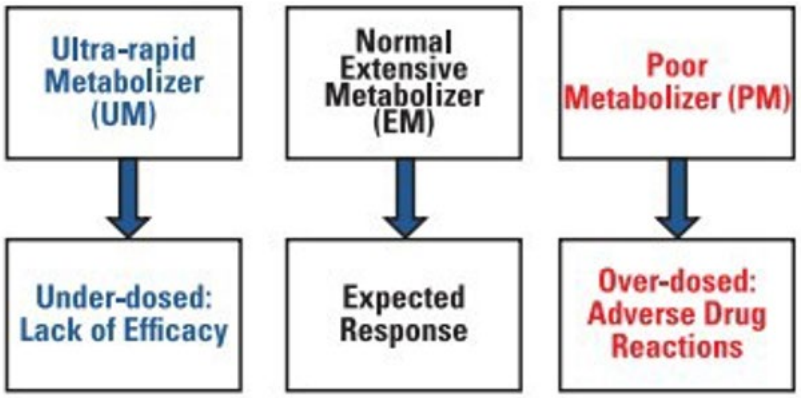


Figure 4: Different phenotypes for polymorphism in drug metabolism.

Precision medicine integrates molecular and clinical information to understand the biological basis of disease. This approach improves the selection of disease targets and has the potential to identify patient populations that will exhibit better clinical results at normal doses.

Figure 6: Representation of percentages of people who experienced ineffective drugs.

VARIATIONS IN DRUG RESPONSE

Clinical responses to administered drugs and subsequent outcomes vary widely across individuals. These patient-specific responses can be inherited or acquired. Such variation presents a challenge in designing an optimal dosage regimen. Statistical studies have revealed that most drugs are effective in just 25%–60% of patients (Ahmed, 2016). Patients are rarely able to reap the full benefits of the initial recommended drug treatment. For example, individuals affected by the following well-known conditions may show no response to primary treatments: depression (38%), asthma (40%), diabetes (43%), arthritis (50%), and cancer (75%) (Ahmed, 2016).

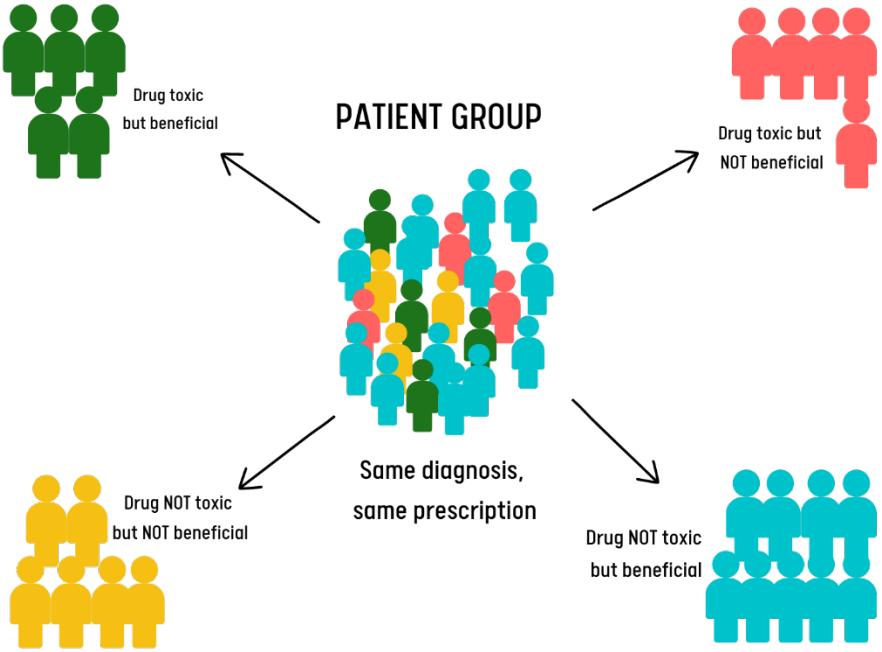


Figure 7:

Different patients can respond differently to the same drug and dose. Sometimes, the effective drug dose for one patient may prove lethal to or result in therapeutic failure for another (too low drug concentrations at normal doses). Therapeutic failure can lead to a serious adverse reaction or no effects at all.

Continuous drug monitoring is recommended when prescribing drugs with known serious side effects and narrow therapeutic indices to avoid unexpected or undesirable outcomes. The risk can be exacerbated if the patient takes other drugs or has another preexisting condition. These factors increase the risk of potentially harmful drug-drug and drug-disease interactions. For example, the daily warfarin dose varies by up to 20- to 30-fold between patients in many disease conditions where it is recommended for the treatment of embolism and thrombosis.

CONTRIBUTING FACTORS IN INTERINDIVIDUAL DRUG RESPONSE

Individual-specific response to medication can be attributed to many multifold and complex factors pertaining to one's unique genetic makeup (mutations such as SNPs, gene deletions, and duplications). These genetic factors can interact with physiological conditions (age, gender, body size, and ethnicity); environmental influences (exposure to toxins, diet, and smoking); and pathological factors (liver and renal function, diabetes, and obesity) to influence drug responses.

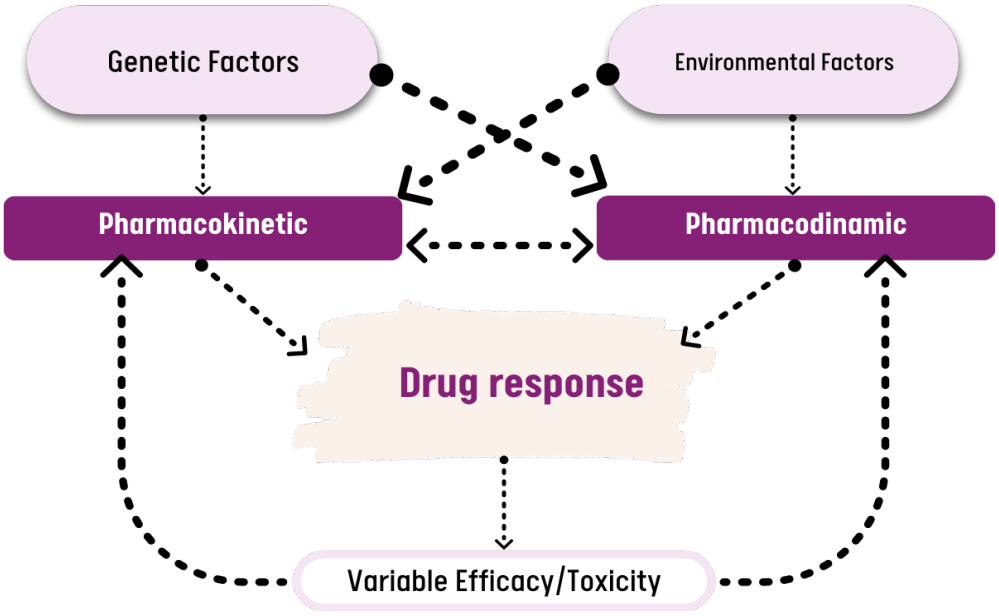


Figure 8

According to the hypothesis of Kalow et al., various genetic factors are 20%–95% responsible for determining variability in response to drugs. Furthermore, individual variations in responses due to genetic factors are often permanent, while those influenced by external factors are mostly transient. In support of inheritance being a major determinant of drug response, Vesel et al. found relatively

higher population variability of a drug response among all the individuals in a population than the interpatient variability at different times.

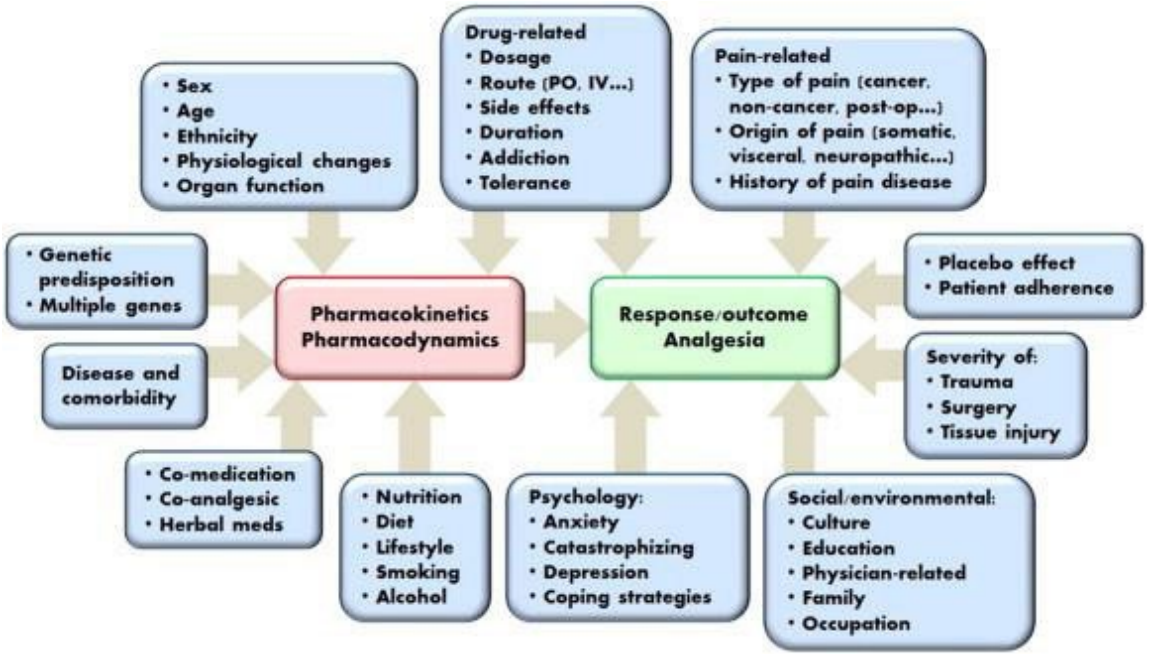


Figure 9

DETERMINANTS OF INTERINDIVIDUAL DRUG RESPONSE

In the past, diseases were diagnosed based solely on symptomology, patient testimonies, and family history. These conditions were only able to be treated once physical or behavioral symptoms set in. At this point, disease pathology could already be well developed and have wreaked irreversible damage.

Following the success of the Human Genome Project (HGP), more specific and precise diagnostic approaches have been developed to examine genes and the genetic variants associated with altered drug responses or specific diseases. Pharmacogenomics enables scientists to assess specific genetic variants that may be responsible for an individual's unique drug response by identifying the exact genetic loci involved (Ahmed, 2016). Whole-genome SNP profiling, haplotyping, multigene analysis, and gene expression studies using biochips or microarrays have recently begun to study individual responses to drugs at various levels. The information gained from such investigations has the potential to promote drug discovery and development.

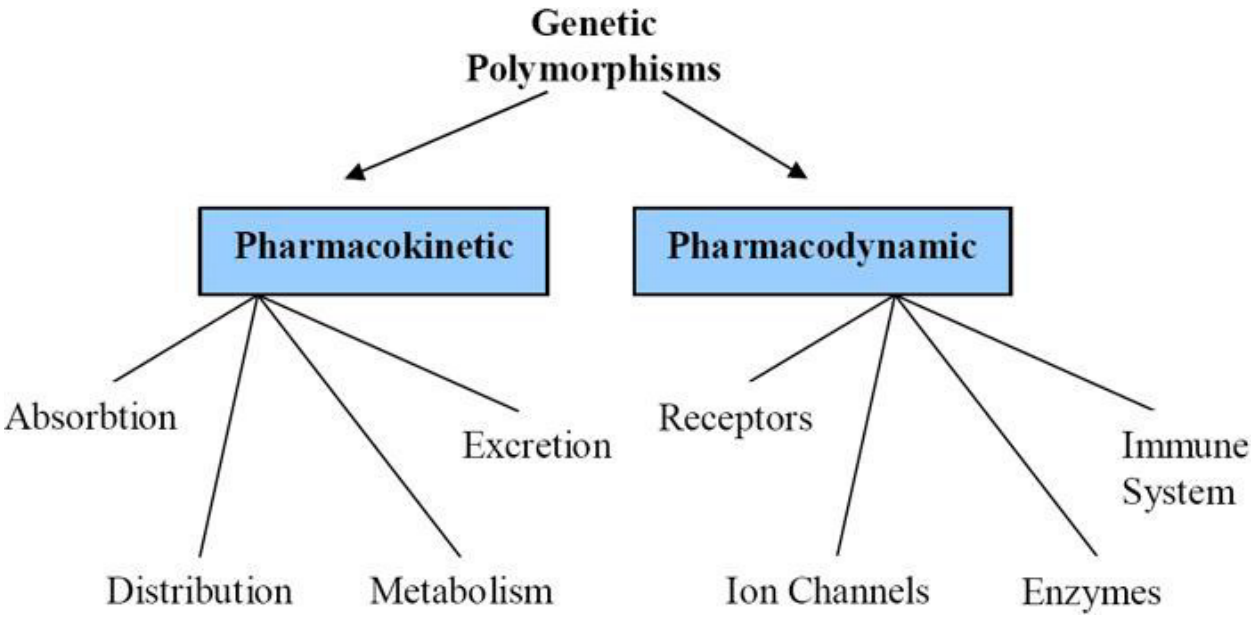


Figure 10:

Genetic polymorphisms may influence a drug's effect by altering its pharmacokinetics and/or pharmacodynamics. Both of which are major determinants conferring the individual differences in drug responses. Pharmacokinetics deals with the dosage required for a to reach its target site. Pharmacodynamics relates to how well the targets (receptors, ion channels, and enzymes) respond to various drugs.

Pharmacokinetics → What the **BODY** does to the **DRUG**

Pharmacodynamics → What the **DRUG** does to the **BODY**

Genetic polymorphisms in drug transporters and phase-1 drug-metabolizing enzymes can alter the pharmacokinetic and pharmacokinetic properties, and/or metabolites of a drug at the target site. These actions cause variability in drug response. Theoretically, variations at a single base (SNPs) or set(s) of closely related SNPs (haplotypes) in the genes involved in pharmacokinetic and pharmacodynamic pathways at any stage can affect an individual's overall drug response.

DESCRIPTION OF PGX TESTING BY P23LABS

Genomic differences between individuals are present approximately every 300–1000 nucleotides and over 14 million single nucleotide polymorphisms (SNPs) are distributed throughout the entire human genome. Additionally, variation in genetic information due to SNPs linked to drug metabolizing enzymes

(DMEs) has been shown to greatly affect drug absorption, metabolism, and excretion of these compounds. Different populations have varied allele frequencies in genes of both drug metabolizing enzymes and transporters. Cytochrome P450 (CYP), which represents a large and diverse group of a heme-containing enzyme superfamily, is involved in the oxidative metabolism of structurally diverse molecules (drugs, chemicals, and fatty acids). Only some dozen enzymes belonging to CYP families metabolize most drugs and xenobiotics (Figure 3)

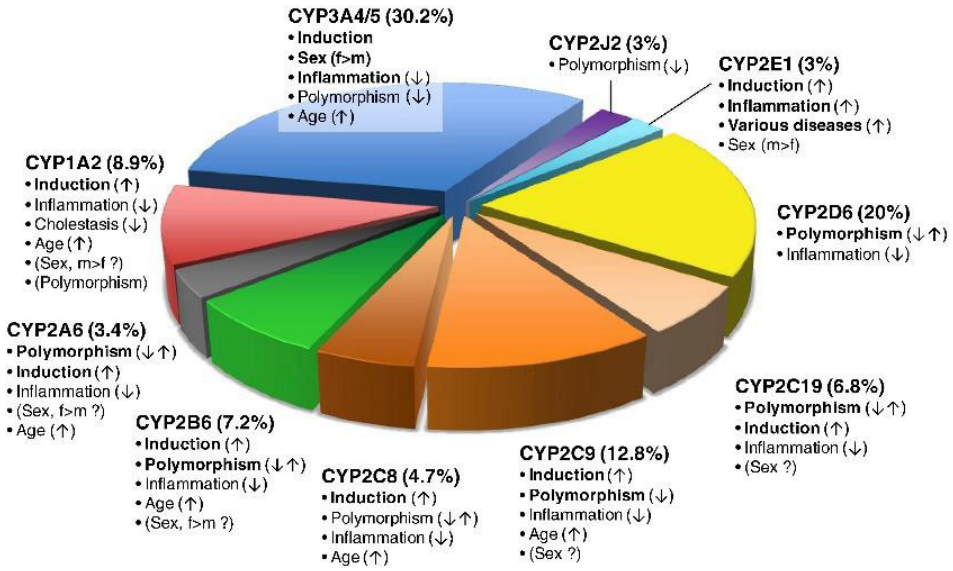


Figure 11: CYP Families Chart

CYP2D6 is the first gene shown to be associated with drug metabolism and has more than 100 variants. The genetic changes of CYP2D6 include point mutations, duplications, insertions, or deletions (including whole gene deletion). Individuals carrying different CYP2D6 allelic variants have been classified as poor metabolizers (PMs), intermediate metabolizers (IMs), extensive Metabolizers (EMs), and ultra-rapid metabolizers (Ums) according to the metabolic nature of drugs and degree of involvement in drug metabolism observed in these variants. The CYP2D6 based phenotypes are shown in the following table. The drugs metabolized by CYP2D6 include tricyclic antidepressants, serotonin reuptake inhibitors, antiarrhythmics, neuroleptics and beta- blockers.

Phenotype	Genotype
PM	*CYP2D6*3–*8, *11,*16, *18–*21, *38, *40, *42, *44, *56, *62*
EM	*CYP2D6*2, *17 x 2, *27, *35, *39, *48*
IM	*CYP2D6*10, *14, *17, *18, *36, *41, *47, *49–*51, *54, *55,*57*
UM	*CYP2D6*2XN* (N = 2, 3, 4, 5 or 13)

Note: Classification is based on the metabolic capabilities of CYP2D6 enzyme on probe substrate (bufuralol, debrisoquine, sparteine, and dextromethorphan) among the sampled individuals in different populations. PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; UM, ultra-rapid metabolizer.

CYP2C9: CYP2C9 is another member of the CYP family and metabolizes approximately 25% of clinically administered drugs including anti-inflammatory agents such as flurbiprofen, hypoglycemics such as

glipizide and tolbutamide, the anticoagulant S-warfarin, and the anticonvulsant phenytoin. More than 60 variant alleles have been identified in CYP2C9 gene. Among them, CYP2C9*2 (R144C) and CYP2C9*3 (I359L) are the most common variants associated with highly reduced CYP2C9 enzymatic activities.

CYP2C19: CYP2C19 can metabolize numerous routinely administered drugs such as anxiolytics (diazepam), proton pump inhibitors (omeprazole), anticonvulsants (S-mephenytoin), and antimalarial biguanides. Up to now, more than 35 CYP2C19 variants and approximately 2000 SNPs have been identified, with continuous increase in SNP numbers reported (http://www.cypalleles.ki.se/cyp2c19.htm). The most common variants, CYP2C19*2 and CYP2C19*3 have been studied extensively. Patients carrying these null variants are categorized as PMs.

CYP3A4 and CYP3A5: More than 50% of clinically administered drugs are metabolized by CYP3A4, the most abundant CYP enzyme in the liver. Therefore, polymorphisms in CYP3A4 are of great concern in studying of interindividual altered drug metabolisms and related adverse drug reactions. Researchers have identified more than 26 CYP3A4 variants, most of which are responsible for varied enzymatic activities (http://www.cypalleles.ki.se/cyp3a4.htm). Impacts range from modest to highly reduced catalytic efficiencies among the affected individuals. CYP3A5 is among the factors that contribute to the complexity of CYP3A4. With few exceptions, CYP3A5 can metabolize most drugs that are substrates of and metabolized by CYP3A4. *CYP3A5* expression is highly polymorphic, with 25 allelic variants of *CYP3A5* (alleles numbered *1-*9). CYP3A4 and CYP3A5 together account for approximately 30% of hepatic cytochrome P450, and hal of medications that are oxidatively metabolized by P450 are CYP3A substrates.

CYP2B6: *CYP2B6* is a member of the cytochrome P450 family of important pharmacogenes and makes up approximately 2-10% of total hepatic CYP content. CYP2B6 is responsible for the metabolism of 4% of the top 200 drugs and is highly inducible by several drugs and other xenobiotics. CYP2B6 is the major enzyme involved in the metabolism of efavirenz and nevirapine. Its pharmacogenomics have become relevant for the treatment of HIV. Variants *CYP2B6*:516G>T and *CYP2B6*:983T>C, as well as haplotypes *CYP2B6*4 and *CYP2B6*6, have been associated with adverse effects of efavirenz treatment.

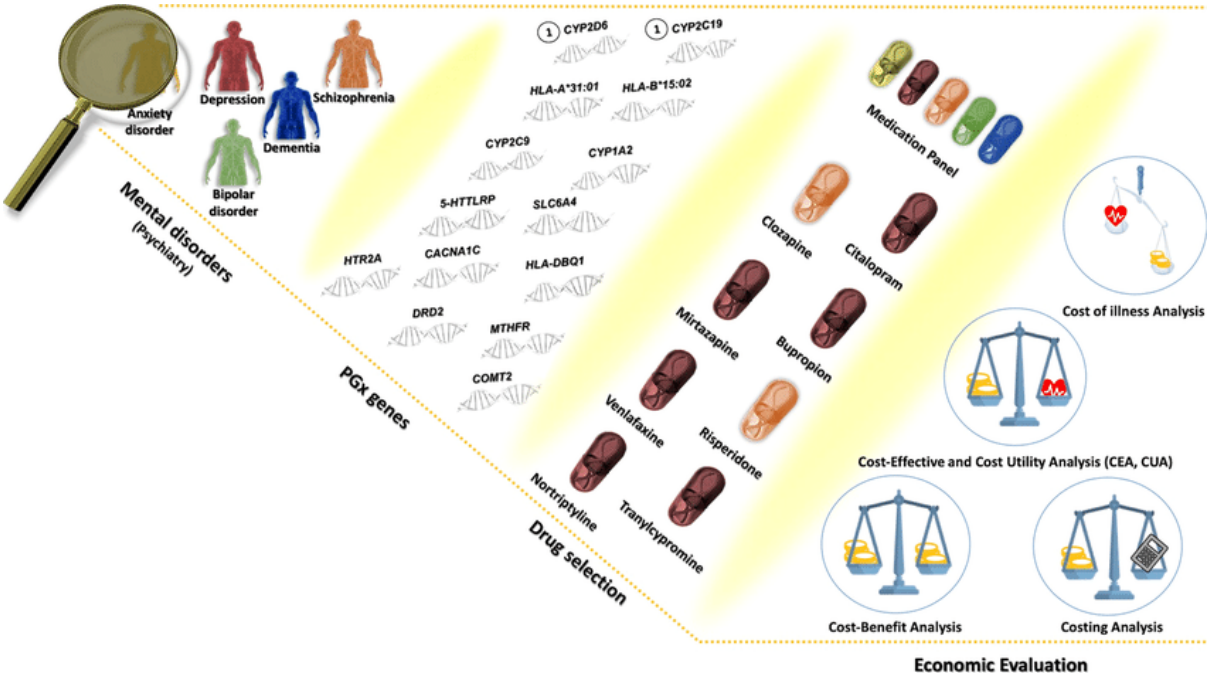
MTHFR: MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the substrate for conversion of homeocysteine to methionine. There are several documented variants in MTHFR. The majority of pharmacogenomic studies focus on MTHFR: 677C>T and MTHFR: 1298C>A. MTHFR is part of pathways that are acted on by several chemotherapeutic antineoplastic and antirheumatic drugs, such as methotrexate and 5-fluorouracil. Due to its relationship with homocysteine, its actions are also relevant to cardiovascular diseases.

VKORC1: The VKORC1 gene encodes the VKORC1 (Vitamin K epoxide reductase) protein, a key enzyme in the Vitamin K cycle. VKORC1 is responsible for the conversion of Vitamin K-epoxide to Vitamin K. This

is the rate-limiting step in the physiological process of Vitamin K recycling. The availability of reduced Vitamin K is of particular importance for several coagulation factor proteins (Factor VII, Factor IX, and Factor) that require it as a cofactor.

VKORC1 is of therapeutic interest both for its role in contributing to high interpatient variability in coumarin anticoagulant dose requirements and as a potential player in vitamin K-deficiency disorders. The examination of VKORC1 variants has shown that polymorphisms in the VKORC1 gene are associated with high and low warfarin dose phenotypes in humans. Overall, VKORC1 polymorphisms account for ~25% of the variance in stabilized warfarin dose. Both the genotypes of VKORC1 and CYP2C9 are clearly the most important genetic factors for warfarin response.

SLCO1B1: The solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene encodes for a membrane-bound sodium-independent organic anion transporter protein (OATP1B1) involved in active cellular influx of many endogenous and xenobiotic compounds. OATP1B1 mediates active transport of endogenous substrates, such as bile acids, xenobiotic compounds, and a wide panel of pharmaceutical compounds. OATP1B1- dependent transport is an important step in mediating drug hepatic clearance. It's role is especially critical within one class of drugs, HMG-CoA reductase inhibitors



(statins), which are widely prescribed for cardiovascular disease (CVD) risk reduction. OATP1B1-dependent transport may also be important for the acid (active) form of simvastatin, (and other statins less hydrophobic than pravastatin) as *SLCO1B1* variants were recently associated with simvastatin-induced myopathies. Thus, implying that OATP1B1 was involved with simvastatin transport.

Factor V Leiden and F2: The Factor V Leiden (FVL) variant (1691G>A; R506Q) in the F5 gene is the most commonly known inherited risk factor for thrombosis. This mutation causes reduced inactivation of

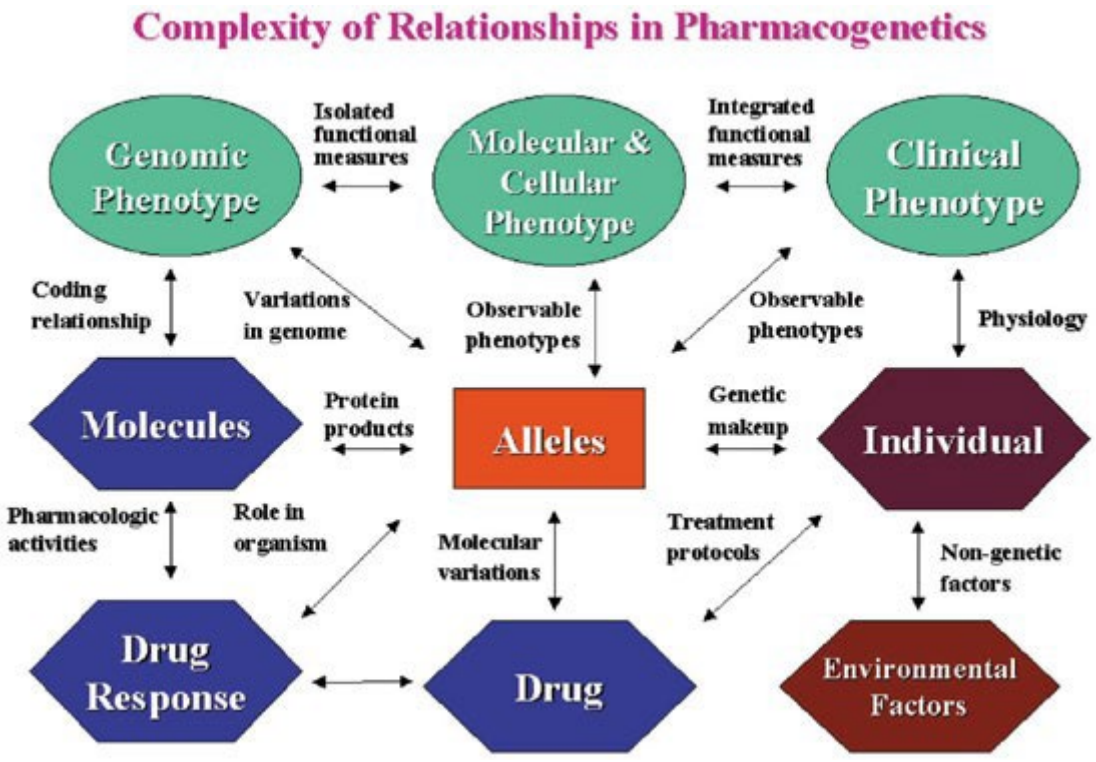
clotting factor V by activated protein C (i.e. APC resistance). This reduction increases generation of thrombin. Heterozygous carriers of the FVL mutation have an approximately 3-fold to 8-fold increased risk of Venous Thromboembolism (VTE) compared to non-carriers. Homozygous carriers of the FVL mutation have a much higher increased risk of VTE (approximately 9-fold to 80-fold). Homozygosity of the FVL mutations is seen in approximately 1/5000 individuals in the general US and European population. Prothrombin (F2) The second most common inherited risk factor for VTE is the 20210G>A (G20210A) variant in the F2 gene. This activating mutation leads to higher circulating levels of prothrombin. This increases the risk for clot formation. Heterozygous carriers of the F2 mutation have a 2-fold to 4-fold increased risk of VTE compared to non-carriers.

CONCLUSION

P23 Labs is on a mission to positively impact the future of national and international genetic disease-related statistics. We endeavor to increase survival rates by providing accessible testing all people regardless of economic status. We aspire to provide patient-friendly products and tests to bring accurate molecular diagnostic results to the general population. These actionable results can empower the patient and enlighten physicians to choose appropriate treatments that improve quality of life.

Our non- or minimally invasive tests are designed to be performed by patients themselves, with little to no professional help. We pledge to provide clear, straightforward results through safe and affordable testing.

In summary, this is the closing argument for the PGx Testing presented by P23 Labs: We believe that following this model and focusing on our mission will help to reduce underutilization and lack of availability to proper disease treatment and personalized genetic testing methods in all, high-risk/at-risk, and under-served populations.



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