Pharmacogenetics and drug metabolism - from rudiments to current for individualised medicine

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ABSTRACT

Background: A developing branch of study that focuses on the genetic investigation of the pharmacogenes responsible for drug metabolism is known as pharmacogenetics.

Objective: This review, focused on how drug metabolism and new pharmacogenetic testing interact.

Methods: A search of existing literature specifically concerned with the use of knowledge gained from the study of gene variations in selected drug metabolising enzymes to direct the use of drugs and associated therapies was carried out. This was with the view of further research in this rapidly developing subject that will help us move away from the "one size fits all" approach to prescribing and improve our knowledge of the factors that influence individual differences in drug disposition and, ultimately, the efficacy or toxicity of medication responses.

Results: Along with improved therapeutic efficacy and public health, potential advantages would include the achievement of better customized prescribing, better patient outcomes in study populations, and more.

Conclusion: This is done to usher in the new era of medical genetics, also known as genetic medicine, which encompasses fields like personalized medicine, gene therapy, and the rapidly developing medical specialty known as predictive medicine.

Keywords: Pharmacogenetics, drug-metabolism, cytochrome P450, genetic polymorphism, genotype tests, personalized medicine.

Pharmacogénétique et métabolisme des médicaments - Des rudiments aux applications actuelles de la médecine individualisée chez les personnes âgées

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RÉSUMÉ

Contexte : La pharmacogénétique est une branche d'étude en développement qui se concentre sur l'étude génétique des pharmacogènes responsables du métabolisme des médicaments.

Objectif : Cette étude s'intéresse à l'interaction entre le métabolisme des médicaments et le nouvel essai pharmacogénétique.

Méthode : Cette étude fait une recherche dans la documentation actuelle qui s'intéresse spécifiquement à l'utilisation des connaissances acquises par l'étude des variations génétiques dans les enzymes métabolisant des médicaments sélectionnés pour orienter l'utilisation des médicaments et des thérapies associées. Cette étude poursuit les recherches sur ce sujet en plein essor qui nous aidera à nous éloigner de l'approche " taille unique " de la prescription et améliorer notre connaissance des facteurs qui influencent les différences individuelles dans la disposition des médicaments et, en fin de compte, l'efficacité ou la toxicité des réponses aux médicaments.

Résultats : Outre l'amélioration de l'efficacité thérapeutique et de la santé publique, les avantages potentiels incluraient la réalisation d'une prescription mieux adaptée, de meilleurs résultats pour les patients dans les populations étudiées, et bien plus encore.

Conclusion : Il s'agit d'ouvrir la nouvelle ère de la génétique médicale, également connue sous le nom de médecine génétique, qui englobe des domaines tels que la médecine personnalisée, la thérapie génique et une spécialité médicale en plein essor, connue sous le nom de médecine prédictive.

Mots-clés : Pharmacogénétique, Métabolisme des médicaments, Cytochrome P450, Polymorphisme génétique, Tests génotypiques, Médecine personnalisée.

INTRODUCTION

Pharmacogenetics and pharmacogenomics - definitions and explanations

Pharmacogenetics, or the study of how genetic variants impact a drug's pharmacokinetics and pharmacodynamic responses-is, the interaction between a medicine and a person's characteristics-is the science of genetic variations in drug pharmacology. In other words, it investigates the relationship between a person's genotype and their capacity to metabolize a foreign substance because of variations brought about by their specific genetic make-up.[1,2] In 1914, Archibald Garrod was the first to link a person's unexpected medication responses to an inability of their enzymes to detoxify foreign compounds, launching pharmacogenetics as an experimental science.[3,4] Werner Kalow also proved heredity as a spectacular result of an unanticipated response to medications in 1962.[5] Genetic polymorphisms in the targets of drug therapy (such as receptors), inherited differences in drug metabolism and disposition,[6,7] individual differences in age, race, organ function, concurrent therapy, drug interactions, and concurrent illnesses are some of the causes of variation in an individual's response to xenobiotics, including pharmaceuticals.[8,9]

The term "pharmacogenetics" emerged to describe the area of study that focuses on the genetic examination of the proteins involved in drug metabolism.[5] Pharmacogenetics is specifically concerned with the use of knowledge gained from the study of gene variations to direct the use of drugs and associated therapies. Two functional component branches of this field link genetics and medicines. These are the pharmacodynamics arm (how medications interact with receptors to produce an expected reaction) and the pharmacokinetics arm (which forecasts how drugs are metabolized by the body). Typically, the biotransformation of medicines by metabolic processes and their subsequent disposal via renal function are closely related to pharmacokinetics. On the other hand, pharmacodynamics focuses on comprehending how medications interact with receptors and the ensuing reaction, even though there may also be some biotransformation involved. Emerging fields like pharmacogenetics and pharmacogenomics concentrate on the genetic factors that influence medication response at the level of individual genes or the complete human genome, respectively. Currently, technologies using gene chip arrays can identify hundreds of differences in a patient's DNA sequence, the majority of which are single nucleotide polymorphisms. Pharmacogenomics seeks to create a profile of DNA sequence variations that are unique to each patient to assess illness risk and choose the best pharmacological therapy. This strategy has the potential to transform disease prevention and treatment[10] using the concept of pharmacogenetic testing.

Pharmacogenetic testing

Thus, pharmacogenetic testing-a relatively new area of clinical and pharmacy practice, particularly in Nigeria-will help in predicting drug concentration or response; achieve better individualized prescribing; improve patient outcomes in the study population; improve therapeutic efficiency and public health.[11,12] When it comes to the metabolism of antimalarial medications, genetic polymorphism is induced by several factors that influence the pharmacokinetics of antimalarial drugs, making this area of pharmacogenetics particularly crucial. This resulted from the discovery of genetic variability in the human metabolism of the anticonvulsant medication mephenytoin.[13] Individuals can be characterized phenotypically as extensive metabolizers (EMs) or poor metabolizers (PMs) of this drug. The enzyme responsible for this polymorphism has been identified as CYP2C19.[14,15] Variant alleles of the human cytochrome P-450 2C19 (CYP2C19; S-mephenytoin hydroxylase) gene have been shown to correlate with the rate at which several antimalarial medications are metabolized. In laboratory medicine, the idea of pharmacogenetic testing is typically related to predicting the biotransformation of a drug by identifying genetic variants that regulate elements of therapeutic response. Pharmacogenetic testing has several benefits, including better prognostication, more accurate diagnosis, identification of clinically distinct patient subsets, easier design of clinical trials, improvement of specificity and safety of current treatments, and identification of disease-defining signaling pathways that result in "druggable" targets.[16] Antidepressants, antipsychotics, warfarin, irinotecan, and tamoxifen prescriptions can now be tested for. There are additional tests planned for beta blockers, anticancer treatments, asthma medications, anti-hyperglycaemics, and hypertensives. (www.healthscopemolecular.com/pharmacogenomics). Existing pharmacogenetic tests or genotype tests for *CYP2C9* & *VKORC1, CYP2C19, CYP2D6,*

and *TPMT* have been prepared on the basis and recommendations of guidelines from various bodies and societies.[17] These bodies include the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline under the National Institutes of Health's Pharmacogenomics Research Network (NIH PGRN), the Dutch Pharmacogenetics Working Group (DPWG) guideline,[18] and the Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice of the National Academy of Clinical Biochemistry (NACB).[19] Other available genotype tests include NAT2 genotype tests for isoniazid, KRAS genotype tests for thiopurine family drugs (azathioprine, mercaptopurine, and thioguanine), HER2 (ERBB2) genotype tests for breast cancer, and EGFR genotype tests for non-small-cell lung cancer.[17] A list of some available tests is found in Table 1.[20]

Table 1: Sample information for some of the currently available tests

Test	Code	Sample Type	Test Frequency
CYP2C9/VKORC1		4mL EDTA blood	Three times/week
CYP2C19 Genotyping	2C19	4mL EDTA blood	Three times/week
CYP2D6 Genotyping	2D6	4mL EDTA blood	Three times/week

Variation in DNA - basis for genetic polymorphism

Venter *et al*.[21] it was who reported from a genome analysis that since we all share at least 99.9% of the nucleotide code in our genome, less than 0.1% of DNA variation accounts for human genetic variability. Patients exhibit varying responses to the environment, differing pharmacokinetics, and predisposition to various situations in clinical and pharmacy practice.[22,23] Hence, they vary regarding dose-response relationships for common drugs and have a range of susceptibilities to adverse effects of therapeutic agents even in the absence of obvious variability in individual pharmacokinetics or biochemical pharmacology.[24] Numerous forms of variation are present in genes encoding drug-metabolizing enzymes, the most prevalent being single nucleotide polymorphisms (SNPs).[25] An SNP is defined as a difference in a single base pair in an individual's DNA, which occurs when one purine or pyrimidine nucleotide is swapped out for another at a specific place in a DNA strand. These can be used to map and pinpoint genes linked to several illnesses, including diabetes, cancer, and arthritis. SNPs exist for many of these genes and are typically biallelic (i.e., involve only 2 choices at a given site within a population). Variant or polymorphism refers to a substitution that is seen in more than 1% of a specific target group but does not manifest any aberrant phenotype.[26] When the protein-coding is unaltered, single-nucleotide polymorphisms can be neutral or change an encoded amino acid or have no effect on gene function.[27] Many of the proteins that these genes encode could end up becoming potential therapeutic targets. On the other hand, a mutation is described as a change in DNA (DNA variant) that happens infrequently, is frequently linked to disease, and may have an impact on phenotypic. Some polymorphisms in genes encoding drug-metabolizing enzymes have been described, though the pharmacogenetic significance of most of these variants is still not fully understood. These polymorphisms may alter enzyme function through changes in gene expression or active-site binding, protein truncation, or yet-to-be-described mechanisms.[28] Variability in the human genome is one of the main reasons why people respond differently to medicines and other xenobiotics. Genetic variation influences nearly every disease vulnerability to some extent and have a substantial clinical impact on drug metabolism. Additionally, genetic variation can affect receptors such as β_2 adrenoceptors, one of which is amplified by salbutamol and results in inadequate control of wheezing and gasping in asthmatics. Others are the 5-HT2A-serotonergic receptor and HER2. Multiple drug resistance transporters are impacted by genetic differences in transporters, which results in phenotypic overexpression in cancer and drug resistance to vinblastine, doxorubicin,

paclitaxel, etc.

Pharmacogenetics and drug metabolism

Drug metabolism[8] was the initial emphasis of the area of pharmacogenetics, but it has since expanded to cover the complete range of drug disposition, drug transporters that affect drug absorption, distribution, excretion, and drug targets.[7,29-30] Drug metabolizing enzymes (CYP450), which are largely found in the liver, play a significant role in determining the therapeutic efficacy of a drug. Instead of being hydrophilic and polar, the majority of orally taken medications are fat soluble and non-polar. After absorption, lipophilic medications go through two-stage biotransformation in the liver. In phase I, they are changed into active or inactive metabolites. However, many medicines and their active metabolites go through second biotransformation (phase II) to make them polar and hydrophilic because excretion ultimately depends on water solubility in urine or faeces. On the other hand, very hydrophilic medications frequently avoid hepatic metabolism and remain mostly unaltered when eliminated in urine. Many medications taken orally are pro-drugs, exerting their full or nearly full pharmacologic impact only on their conversion to active metabolites (e.g., proguanil hydrochloride to cycloguanil) and the less potent metabolite 4-chlorophenyl biguanide. The metabolism of drugs in humans is carried out by more than 30 families of enzyme complexes.[31,32] and these all have been found to exhibit genetic variation; many of which translate into functional changes in the respective proteins encoded.[9] These drug-metabolizing enzymes are categorized as catalyzing either phase I (oxidation, reduction, and hydrolysis) or phase II (conjugation, acetylation, glucuronidation, sulphation, and methylation) reactions. They work to transform relatively lipid-soluble substances into water-soluble metabolites that are easily excreted. The cytochrome P450 microsomal enzymes, a group of heme-containing proteins that catalyze the transformation of lipophilic compounds into hydrophilic molecules that can ultimately be eliminated by kidneys in urine, catalyze the majority of significant phase I processes. It represents a major part of the body's powerful detoxification systems localized primarily in hepatocytes but also in the intestines.[33] The cytochrome P450 system undergoes several processes, such as epoxidation, N-dealkylation, O-dealkylation, S-oxidation, and hydroxylation, to metabolize both endogenous and foreign substrates.

Cytochrome P450 and genetic polymorphism

Humans have a multigene family of drug-metabolizing enzymes called cytochrome P450s (CYP450), which are primarily present in the liver and oversee the metabolic elimination of most pharmaceuticals now utilized in medicine.[34] They are members of a family of isozymes that are found in the endoplasmic reticulum and are involved in the oxidative metabolism and biotransformation of drugs. They are crucial for the biosynthesis and breakdown of many endogenous substances, including those with still-unknown functions like drugs, foreign substances, arachidonic acid, and eicosanoids. They also play a crucial role in the metabolism of cholesterol and bile acids, steroid synthesis and metabolism, vitamin D3 synthesis and metabolism, steroid biosynthesis, and steroid metabolism. Mutations in many CYP genes cause inborn errors of metabolism and contribute to many clinically relevant diseases. The human genome thus contains 18 CYP families, divided into 41 protein-coding subfamilies encoding 57 genes,[35] but only a relatively small number of the encoded proteins, mainly in the CYP1 (A1, A2, B1), CYP2 (A6, A13, B6, C8, C9, C19, D6, E1, F1, J2, R1, S1, W1), and CYP3 (A4, A5, A7, and A43) families, appear to contribute to the metabolism of drugs. The CYP 4 family has also been reported as part of the PharmVar Genes (https://www.pharmvar.org/genes). More than half of all medications are largely cleared by the cytochrome p450 mixed-function mono-oxygenase system, which is likely the most significant component of phase I metabolism in mammals. These enzymes are sometimes referred to as drug-metabolizing enzymes (DME), and several variables, including age, food, concurrent drugs, and genetic variability, affect how active they are. Between 20 and 200 medications are thought to oxidize cytochrome P450 isozymes.

Only 6 isoforms catalyze the oxidative metabolism of most drugs in common use: CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP2E1.36 Of these six isozymes, the CYP3A4 isozyme's common metabolism has led to several clinically relevant drug-drug interactions. Particularly, CYP3A, CYP2D6, and CYP2C19 are those responsible for over 50% of the overall clearance of regularly used medications and around 80% of oxidative drug metabolism. Although there may be significant overlap, each cytochrome P-450 enzyme has a distinct substrate specificity that is frequently to a specific area of a drug molecule, to a specific enantiomer (such as for S-mephenytoin), or both. As a result, one cytochrome P-450 enzyme may be substantially in charge of the entire oxidative metabolism of a certain drug, or several cytochrome P-450 enzymes may each contribute. The liver is the predominant site of cytochrome P-450-

mediated metabolism, but the enterocytes in the epithelium may contribute.[37] While some medications are substrates for multiple enzymes, others are substrates for just one. The medications that are most likely to be engaged in clinically significant drug interactions are those that have long-lasting effects by acting on enzymes that metabolize other substrates. Every cytochrome P450 isozyme has a unique gene that codes for it, and the P450 gene superfamily is divided into families and sub-families according to how closely related its isozymes are to one another in terms of amino acids. Cytochrome P-450 enzymes reduce or alter the pharmacologic activity of many drugs and facilitate their elimination. Individual cytochrome P-450 enzymes are classified by their amino acid similarities[33,38] and are designated with Arabic numerals by a family number, a subfamily capital letter, a number for an individual enzyme within the subfamily, and an asterisk followed by a number and a letter for each genetic (allelic) variant e.g., CYP2C19 (www.cypalleles.ki.se).

Mechanisms for cytochrome P450-mediated drug interactions

The two main mechanisms behind cytochrome P450-mediated drug interactions are induction and potent inhibition. Increased production or decreased degradation of cytochrome P450 enzymes are referred to as induction; these effects promote conversion to inactive metabolites. Therefore, induction causes a drop in the substrate's plasma levels as well as the pharmacodynamic impact. Either enzyme inactivation or reciprocal competition between substrates for a catalytic site is considered a form of inhibition. Both responses have the same overall result of slowing down drug metabolism, which lengthens the half-life of the affected medication or active metabolite and intensifies its pharmacologic (or toxic) effect. Examples of inhibitors include cimetidine and fluconazole, whereas rifampicin functions as an inducer. It has been suggested that variations in the activity of these enzymes are responsible for the inter-individual diversity in drug responsiveness and toxicity. A detailed cytochrome P450 "Clinically Relevant" Drug Interaction Table for substrates, inhibitors, inducers, and genetics for the CYP 450 enzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5 and 3A7 can be assessed at the clinical pharmacology home page of the Department of Medicine, Indiana University (http://medicine.iupui.edu). While the frequency of mutant alleles of CYP3A, 2D6, 2C9, and 2C19 has been studied in all major human races, scanty or no data are available for Nigerians.

Cytochrome P450 CYP3A and drug metabolism

The CYP3A family of cytochrome P-450 enzymes together describe metabolism that is mostly carried out by two enzymes, CYP3A4 and, to a lesser extent, CYP3A5, whose substrate specificities are so close that they cannot be easily identified. Although Cytochrome P450 3A4 (CYP3A4) is the most prevalent and important drug-metabolizing enzyme among the Cytochrome P450s, no homozygous inactive variation has been identified yet.[38] However, 37 variant alleles of the CYP3A family have been identified to date (i.e., CYP3A4*1 to CYP3A4*37).[39-42] https://www.pharmvar.org/gene/ CYP3A4). CYP3A4 undergoes extensive metabolism in the intestinal mucosa and the liver which contributes to the low oral bioavailability of many drugs.[33] The CYP3A activity may be reduced by drug interactions through inhibition, or it may increase metabolic activity through an induction which can expand the range of variability to about 400-fold.[43,44] As demonstrated by the reported interaction between erythromycin, a medication that is substantially metabolized by CYP3A, and inhibitory medications such nitroimidazole antifungal medicines, diltiazem, and verapamil, among others, the problem of drug interactions can be problematic. A patient using both erythromycin and one of the inhibitors may have an increase in erythromycin levels. Because erythromycin prolongs cardiac repolarization, unexpected death could result.[45] When CYP3A is inhibited, an oral medication that undergoes significant first-pass metabolism may have its bioavailability multiplied. Other powerful CYP3A inhibitors are known to raise the plasma concentrations of medicines processed by CYP3A enzymes even when administered at standard doses. Unless the dosage is changed, adverse consequences are predictable. However, medications that block CYP3A activity can occasionally be used with other protease inhibitors to treat HIV type 1 infection. This is the case with ritonavir, which is the basis for this strategy. Certain inhibitors of the HIV-encoded protease have a greatly reduced first-pass metabolism when administered with ritonavir, and their plasma levels have significantly increased as a result. Following the discontinuation of the interfering medication, a reversible CYP3A inhibition is often seen within two to three days. However, because CYP3A is destroyed and a new CYP3A enzyme must be produced, the impact may continue significantly longer in the case of inhibitors, including diltiazem, macrolide antibiotics, mifepristone, and delavirdine.[43] Tacrolimus is a substrate for CYP3A4[39] and its co-administration with CYP3A4 inhibitors such as diltiazem causes clinically significant toxicity while CYP3A4 inducers like carbamazepine

reduce tacrolimus concentrations. Grapefruit juice as a non-drug, inhibiting CYP3A4 increases the concentrations of many drugs.[46]

Cytochrome P450 CYP2C subfamily and Genetic Polymorphism

Four members of this family: CYP2C8, CYP2C9, CYP2C18, and CYP2C19 have been described. They are encoded by four highly homologous genes on chromosome 10, each isoform having very distinctive substrate specificity that subjects them to individual consideration of their polymorphic consequences.[47] The cytochrome P450 2C subfamily is also a key player in the generation of epoxyeicosatrienoic acids (EET acids) and CYP2C9 and CYP2C19 are the major CYP2C involved in 8, 9-EET, production[48] (a major CYP450 metabolite in the renal cortex) that has anti-inflammatory properties.[49]

Cytochrome P450 CYP2C8

CYP2C8 officially named cytochrome P450 family 2 subfamily C member 8, is exhibited as the wild-type allele in addition to at least three variant alleles with nonsynonymous base substitutions.[50-52] It was one of the first human cytochromes P450 with a drug metabolic function to be cloned.[53] About eighteen variant alleles of CYP2C8 have been identified.[54]
(https://www.pharmvar.org/gene /CYP2C8). Substrates for CYP2C8 with large interindividual differences in its enzymatic activity include anticancer drug paclitaxel, all-trans retinoic acid, arachidonic acid, cerivastatin, rosiglitazone, zopiclone, and the antimalarial drug amodiaquine.[52,55] However, its inhibitors include gemfibrozil and ketoconazole. The variant alleles, CYP2C8*2, CYP2C8*3, and CYP2C8*4 are associated with decreased activity when paclitaxel was used as substrate in comparison with the wild-type allele.[56] CYP2C8*2, CYP2C8*3, and CYP2C8*4 occur at a frequency of very rare, 0.13 and 0.075 in Europeans; but at 0.18, 0.02, and very rare respectively in African Americans.[57] Other variant alleles are CYP2C8*5 to CYP2C8*18.[54] However, work is ongoing on the systematic collection of the CYP2C8 allele frequencies and the systematic curation of the function of CYP2C8 alleles by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (https://www.pharmvar.org/gene/CYP2C8).

Cytochrome P450 CYP2C9

Several clinically significant medicinal medicines are metabolized by the enzyme CYP2C9, which has been shown to have several single nucleotide polymorphisms. Non-steroidal anti-inflammatory drugs (NSAIDs i.e., "profens"), phenytoin, sulfonylureas, and warfarin are substrates for CYP2C9, while fluconazole and sulphaphenazole are inhibitors. The pharmacokinetics and pharmacodynamics of many therapeutic medications are greatly influenced by CYP2C9 in this function, which may lead to negative pharmacological effects and therapeutic failure. In a bacterial cDNA expression system, their allelic expression revealed that multiple alleles had changed catalytic activity.[58,59] Although CYP2C9 allele distribution varies by ethnicity, the overall frequency of variant alleles in the general population appears to be around 30%.[60-62] Each of the two most prevalent variant CYP2C9 alleles (CYP2C9*2 and CYP2C9*3) is known to be at least as common in a range of Caucasian groups as the HFE (hemochromatosis) gene mutation that results in the substitution C282Y.[60,61,63] The HFE gene has two common mutations, C282Y and H63D which can be revealed by genetic testing. However, heterozygosity for mutant CYP2C9 alleles has been linked to discernible changes in clinical phenotype, unlike C282Y. Higashi *et al*.[60] have shown longer time to correct dose (to administer the correct amount), and increased frequency of bleeding events. A correlation with changed dosing requirements has been shown in a few studies.[61,62,64] a Swedish population, it was discovered that 29% of the variation in the maintenance warfarin dose can be linked to the CYP2C9 genotype. Hillman *et al*.[61] found that a gene-based multivariate model with clinical variables could account for 34% of the variance (for example, age, gender, and body size). The functional effects of the CYP2C9*5, CYP2C98*6, CYP2C9*8, and CYP2C9*11 polymorphisms were also examined in vivo among black Africans, where 19 Beninese participants received a single oral dose of losartan (25 mg).[65] They concluded that, in contrast to the wild-type variant, the CYP2C9*5 and CYP2C9*6 alleles are linked to lower enzyme activity in vivo, whereas the CYP2C9*8 and *11 variants did not seem to have significant in vivo impacts.[65] The warfarin-CYP2C9 relationship represents a well-characterized example of single DME polymorphism predisposing patients to the development of a clinically recognizable alteration in phenotype.[60,61,66] The limited therapeutic index of warfarin is responsible for the phenotypic penetrance of this association. It is advised that CYP2C9 and VKORC1 genotype tests be carried out to ensure that warfarin is dosed properly for each patient.[17] About eighty-five variant alleles of CYP2C9 have been identified in various study populations.[54,58,65-70]
(https://www.pharmvar.org/gene/CYP2C9).

Cytochrome P450 CYP2C19 and genetic polymorphism

A CYP database (http://www.cypalleles.ki.se/cyp2c19.htm) reports that there are currently over 34 CYP2C19 variant alleles, including unusual gene deletions. CYP2C19 is a highly polymorphic gene. It has been established that CYP2C19*35 is the most recent addition to the CYP2C19 gene, which is located on chromosome 10q24.[71] (https://www.pharmvar.org/gene/CYP2C19). The frequencies of these variant alleles differ significantly across ancestrally diverse populations (CYP2C19 Allele Frequency Table online.[72], CPIC: https://cpicpgx.org/). Functionally, alleles are categorized into different groups including normal function (e.g., CYP2C19*1), decreased function (e.g., CYP2C19*9 and CYP2C19*10), no function (e.g., CYP2C19*2 and CYP2C19*3), and increased function (e.g., CYP2C19*17).[72] CYP2C19 is a well-known genetic polymorphism in the metabolism of the anticonvulsant drug mephenytoin in humans[13] which has been attributed to defective CYP2C19 alleles. Individuals can be classified as EMs or PMs of this drug phenotypically. With the poor metabolizer (PM) phenotype representing 2-5% of Caucasians and 13-23% of Oriental groups, this genetic polymorphism exhibits severe interracial disparities. According to reports, Indian populations (North Indians (NI) 33.1%; South Indians (SI) 36.8%) have a greater prevalence of the CYP2C19*2 allele than African (16%), Caucasian (13.3%), or Asian (28.4%) groups.[73] This polymorphism affects the metabolism of some other clinically used drugs such as the antiulcer drug, omeprazole[74], certain barbiturates,[75,76] and antidepressants e.g., imipramine,[77-80] the antimalarial proguanil,[81] and to a lesser extent the β-blocker propranolol,[82] and the anxiolytic diazepam.[83] The enzyme responsible for this polymorphism has been identified as cytochrome P450 2C19 (CYP2C19).[14,15] Several polymorphisms of the CYP2C19 gene have been identified and these produce an inactive enzyme.[84] The majority of poor metabolizer (PM) phenotypes are caused by two variant alleles, CYP2C19*2 and CYP2C19*3, which have G-to-A nucleotide substitutions in exon 5 and exon 4, respectively, resulting in abnormal splicing sites and a premature stop codon, respectively.[85] CYP2C19*3 is primarily present among Orientals, even though CYP2C19*2 seems to be the allele most frequently related to the PM phenotype.[86] In the majority of populations that have been researched thus far, the CYP2C19*2 and CYP2C19*3 alleles account for more than 95% of the defective alleles.[87] Any of these heterozygous alleles require a change in medication dosage.[88,89] The significant inter-subject variability in CG concentrations in humans may be explained by the crucial role played by the P450-isozyme (CYP2C19) in the polymorphic oxidation of mephenytoin. According to the research by Ward *et al*.[81], this phenotype lacks or has a diminished antimalarial impact on PG. Additionally, omeprazole was found to increase the proguanil to cycloguanil metabolic ratio in urine in a prior study based on an analysis of these ratios[90]. This result is consistent with the inhibition of cycloguanil formation and not only confirms the interaction but also aids in identifying its potential mechanism and predictors. The biotransformation of proguanil into cycloguanil, which is known to be metabolized by both CYP2C19 and CYP3A4.[74,91-93] was likewise found to be inhibited by omeprazole in vitro and in vivo. In patients with the CYP2C19 extensive metabolizer phenotype, the clinical implications of the reduction in cycloguanil production in the presence of omeprazole suggest that protection against malaria may be lowered when omeprazole and proguanil are combined. Additionally, CYP2C19 breaks down endogenous arachidonic acid to create epoxyeicosanoid acids, which have a role in inflammation and vascular tone. Consideration may be given to CYP2C19 as a new candidate gene for cardiovascular risks brought on by inflammation because of the correlation between the concentration of inflammatory markers and the CYP2C19*2 polymorphism.[94] Additionally, it was discovered that there are significant genetic and phenotypic variations in plasma levels of proton-pump inhibitors, which are mirrored in changes in gastric pH brought on by the drugs.[95] As a result, the healing rate for both stomach and duodenal ulcers displays a CYP2C19 gene dose effect, and the cure rate for Helicobacter pylori infection when a proton-pump inhibitor and amoxicillin are taken is dependent on the CYP2C19 genotype.[96,97] Clopidogrel, an antiplatelet prodrug, is bioactivated by the enzyme CYP2C19, and common CYP2C19 loss-of-function alleles are linked to harmful cardiovascular events.[98] In linkage disequilibrium with CYP2C19*17, the loss-of-function allele CYP2C19*4 was also discovered. When analysing CYP2C19*17, this important haplotype, known as CYP2C19*4B, changes how CYP2C19 genotyping is interpreted. Furthermore, the prevalence of extensive metabolizers decreased from 70% to 40% because of genotyping CYP2C19*17, and 30% were reclassified as ultrarapid metabolizers.[98] The three cytochrome P450 enzymes with the closest ties to clinical applications through pharmacogenetic testing are CYP2D6, CYP2C9, and CYP2C19. The poor metabolizer phenotype of CYP2C19 is thought to have reduced clearance for some medications, which could lengthen or intensify the pharmacological impact.[99,100] As a result, people with poor drug metabolizers might only need

smaller doses of medications like diazepam.[83] Poor metabolizer and extensive metabolizer phenotypes in the case of proguanil may have different side effects or toxicity profiles.[101] Figure 1 (adapted) shows metabolizer status, and influence on drug dosing in studies of genetic variability.[20] This information is provided in accordance with the National Academy of Clinical Biochemistry's (NACB) Laboratory Medicine Practice Guidelines. The four phenotypic categories of ultra-rapid, extensive, moderate, and poor metabolizers have previously been used to categorize this heterogeneity.[20] However, the CYP2C19 Genotype or (Diplotype)-Phenotype Table online can be consulted for a comprehensive list. The CYP2C19 Allele Functionality Table and the CYP2C19 Allele Frequency Table online can also be consulted for allele functions and population-specific allele and phenotype frequencies,[72,102] respectively, for specific CYP2C19 genetic test interpretation (PharmVar.https://www. pharmvar.org/gene/CYP2C8). Inhibitors of CYP2C19 include fluoxetine and ketoconazole.

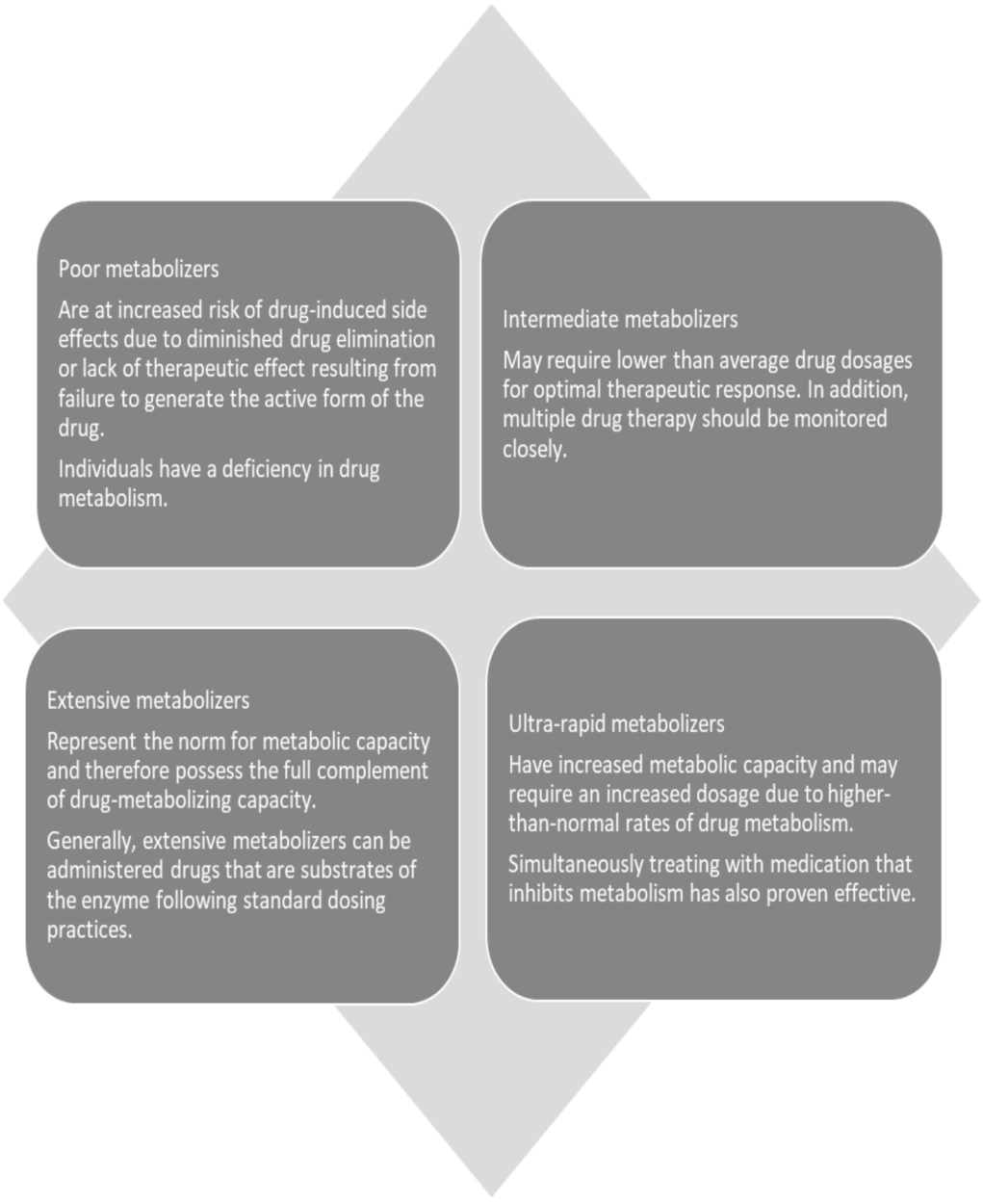

Figure 1. Metabolizer status, and influence on drug dosing in genetic variability studies. (Adapted from 22)

DISCUSSION

Cytochrome P450 CYP2D6 and genetic polymorphism

CYP2D6 is a key enzyme for drug bioactivation and excretion, whose activity is primarily controlled by genetic variation.[103] It has been demonstrated that the activity of CYP2D6, also known as debrisoquine hydroxylase, is highly polymorphic and affects the pharmacokinetics and pharmacodynamics of several drugs. There are approximately 163 variant alleles known (CYP2D6*1 to CYP2D6*15; CYP2D6*17 to CYP2D6*163).[104] (Gene ID: CYP2D6 @ https://www.pharmvar.org). A test for big gene deletions/duplications, such as a long PCR or multiplex ligation-dependent probe amplification (MLPA), is advised to identify the CYP2D6 genotype and should be carried out concurrently with a test for single-nucleotide variations.[17] The diversity of CYP2D6 substrates includes medications for the central nervous system (CNS), the heart, analgesics, and hormones such as tamoxifen, tricyclic antidepressants, neurotransmitter reuptake inhibitors, neuroleptics, and neurotransmitter reuptake inhibitors.[105-111] Additionally, CYP2D6 metabolizes other drugs such as perhexiline and phenformin and some environmental toxicants. Most preferred substrates include an alkyl or aryl amine. CYP2D6 was the first genetic polymorphism to be identified.[112] This enzyme is important in the clearance of numerous drugs, and its ability to do so can vary by 200-fold.[113] There are several potentials for drug-drug, drug-host, and drug-environment interactions for CYP2D6 because its' activity is influenced by the host's genetic makeup and environmental/medicinal exposures. More and more experts in the field are realizing the significance of the CYP2D6 genotype in (a) assessing pharmacological efficacy, (b) determining the likelihood of adverse drug reactions, and (c) creating patient-specific dose levels.[114,115] A few genetic variants that underpin the CYP2D6 metabolizer phenotypes of poor, moderate, extensive, and ultrarapid abilities have been identified by gene probe research. The PM group has the *3, *4, *5, or *6 alleles, all of which code for a protein that has decreased or null CYP2D6 activity. The EM group carries the wild-type (*1) or active (*2) variant alleles.[116] In northern Europeans, gene duplication is relatively uncommon, although it can happen in as many as 29% of people with north-eastern African ancestry. Carriers of two non-functional alleles for CYP2D6 and CYP2C19 are referred to as poor metabolizers because they have a highly decreased ability to metabolize medications that are substrates for these enzymes. Additionally, for 2D6 and 2C19, groups of various racial origin exhibit significantly varying prevalence of loss of functional alleles or alleles encoding for enzymes with decreased activity.[117] Caucasians (5 to 10 %) have a poor ability to metabolize (homozygous for null variants), as do Southeast Asians (1-2 %) which in turn disposes them to the risk of compromised metabolism or adverse drug reactions when prescribed with medications that are substrates of CYP2D6. Certain alleles have equally been discovered only in particular racial/ethnic groups,[118] as reported for CYP2D6*17 which has lower activity, in Black Africans,[119] while CYP2D6*10 (which similarly confers reduced activity) is widespread among Southeast Asians but not among other populations.[116] Around 25% of currently given medications, such as different antidepressants, neuroleptics, beta-blockers, opioids, antiemetics, and antiarrhythmics, are metabolized by CYP2D6, this highly polymorphic pharmacogene. As a result of the high plasma concentration of the affected drug in patients with poor metabolism and the resulting increased risk of adverse reactions, as well as the consequently low plasma concentration of the affected drug,[37] in patients with ultrarapid metabolism, CYP2D6 polymorphisms are clinically significant. Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, paroxetine, and fluvoxamine, are CYP2D6 inhibitors in addition to quinidine. Codeine cannot be metabolized to morphine when given to a patient taking any of these medications, which prevents it from having any analgesic effects.[113] Due to a lack of the active opiate moiety, those with CYP2D6 deficiencies may not get pain relief from codeine, while people with faster metabolism may have more frequent peaks of the active opiate with accompanying side effects.[120] Various studies using debrisoquine, metoprolol, sparteine, and dextromethorphan as CYP2D6 substrates have investigated the prevalence of CYP2D6 poor metabolizers in the African population including African-American children[121] Ghanaians,[122] Nigerians,[123-125] southern Africans (Burundians,126 Barakwena Bushmen,[127] Venda,[128], Zimbabweans,[129]) as reviewed by Bradford and Kirlin.[130] An investigation into the frequent distribution of ultrarapid metabolizers of debrisoquine in an Ethiopian population has also been documented,[131] as well as CYP2D6 genotype predictions of plasma concentrations of tamoxifen metabolites in Ethiopian breast cancer patients.[132]

Genetic diversity of African populations

The genetic diversity in African populations has been researched and reviewed in published literature.[133] In their review, they concluded that CYP polymorphisms clearly demonstrated and confirmed that genetic

variation is greater in African populations than in Asian and Caucasian populations. Because genetic variability in genes encoding drug-metabolizing enzymes may play a role in the widespread reporting of adverse drug reactions across Africa, the African continent cannot be treated as a homogenous, single entity in drug research and development. Neither can African American populations stand in as an adequate proxy for pharmacogenetic differences across Africa.[133] Their review also indicated that population genetic studies have been done in Benin republic, Democratic Republic of Congo, Kenya, North Sudan, Tanzania, and Uganda amongst others.[133]

Techniques in genetic polymorphism

Phenotyping

Principle of phenotyping

Based on the injection of a probe drug that is metabolized by this enzyme to a metabolite that can be identified in urine, cytochrome P450 expression in patients is phenotyped.[134] Hence, phenotyping has been done using both urine and plasma concentrations of drugs and their metabolites. The basic idea is to first compute the substrate's urine or plasma concentrations before calculating the metabolic ratio between the parent medication and its metabolite. Dextromethorphan, sparteine, and debrisoquine have all been used as phenotyping probe medicines to measure CYP2D6 activity. The CYP2C19 substrate, S-mephenytoin, was originally employed to phenotype individuals for CYP2C19 activity. Due to adverse side effects of S-mephenytoin,[135] proguanil (PG) has been used as an alternative and safer CYP2C19 probe drug for phenotyping purposes in Caucasians,[81] Thais,[136] Vietnamese,[137] Turks,[138] and Tanzanians,[139,140] Kenyans,[141] Nigerians,[142,143], etc. with comparable results to those obtained from mephenytoin. This is also a result of the challenges in administering mephenytoin and measuring its oxidative metabolite in urine. Since proguanil and omeprazole are substrates of the CYP2C19 subtype that also metabolizes mephenytoin and that their metabolism co-segregates with that of mephenytoin, proguanil might theoretically also substitute mephenytoin for phenotyping. Additionally, due to its use as an antimalarial chemoprophylactic, proguanil is more widely available throughout Africa, Asia, etc. In numerous population studies, the ratio of PG to CG content in urine has been utilized as an indicator of inter-individual variability.[82,91] According to these studies, the population distribution in this area is significantly skewed, with a small percentage of people producing tiny amounts of CG and a consequently large urinary ratio. The rate and extent of PG to CG metabolism have been attributed as reasons for the variability in the PG/CG ratio and individuals have been characterized as extensive (PG/CG ratio < 10) or poor (PG/CG ratio > 10) metabolizers of PG.[91] The ability of a drug to competitively inhibit the oxidation, and to implicate the matching isoenzyme in the metabolism of the specific drug results in a deficiency in a particular enzyme when more of the parent drug and less of the metabolite are removed.[91] Therefore, mephenytoin, omeprazole, and proguanil are probe drugs for CYP2C19 phenotyping.

HPLC in phenotyping

Methods for genotyping[144] and phenotyping[142,143] can be used to identify polymorphisms. The process of phenotyping involves giving probe medications and then measuring the metabolic ratio. On the other hand, DNA must be extracted to do genotyping. The linearity, accuracy, precision, sensitivity, and specificity of the chromatographic method, HPLC, make it the optimum approach for analyzing probe drugs and their metabolites.[145] According to Kobayashi *et al*.[146], omeprazole and its two major metabolites, 5-hydroxyomeprazole, and omeprazole sulfone, were measured by HPLC. High-performance liquid chromatography with UV detection followed by solid phase extraction is the most effective technique for proguanil analysis.

Phenotyping

Omeprazole and its CYP2C19 produced 5-hydroxylated metabolite or proguanil and its active metabolite, cycloguanil, are measured in urine or plasma, respectively. In accordance with typical Phase 1 standard controlled circumstances, subjects are given a single 20 or 40 mg omeprazole capsule or a 100 or 200 mg proguanil tablet. For PG, urine is collected up to 8 hours after medication administration, and it is subsequently analysed for drug and metabolite concentrations.[81,82,91,136,141] At three or four hours after the dose, one plasma sample can be taken to test for PG and metabolite. Next, the metabolic ratio is determined. Only one plasma sample is taken 2 or 3 hours after the omeprazole dose, or plasma can be taken from drug intake up to 24 hours after dosing.[147] Proguanil and omeprazole assays are often carried out using HPLC and UV detection[143,148-151] or LC-MS/MS assays.[152,153] Time-dependent kinetics of omeprazole limits its use for phenotyping during chronic therapy.[154] Also, the use of omeprazole in CYP2C19 phenotyping may be affected by

liver disease, age, and omeprazole therapy.[155]

Genotyping

Principle of genotyping

The more frequently measured phenotype is the result of a person's drug metabolism, whereas the genome represents a person's complete gene structure. However, the phenotype is not always consistent with the genotype because it is the outcome of interactions between genetic make-up and environment. As genotype determination technology develops, more precautions will be needed to ensure accurate and reliable test findings. The laboratory shall adhere to the international standards set forth for molecular pathology testing when doing pharmacogenetic testing.[156] The extracted nucleic acid's quality and size criteria will therefore be determined by the pharmacogenetic test procedure. Larger sizes of nucleic acids with little degradation are needed for southern analysis. For various sources of nucleic acids, several enzymatic amplification techniques may be used for genotype characterization. Nucleic acid extraction procedures should adhere to molecular pathology guidelines.[157] When doing pharmacogenetic testing in a laboratory, there are standard operating procedures and rules to follow, albeit these procedures will vary on the location of the laboratory. However, other international laboratories may accept and adhere to the College of American Pathology (CAP) criteria for the United States, or the ISO 15189 guidelines provided by the Technical Committee of the International Organization for Standardization (http://global.ihs.com).[158] The answers to queries on the materials to be used to validate pharmacogenetic tests, the techniques used to prevent or detect assay interferences, and the material to be used for validation and lot-to-lot quality control will be provided by this guideline. https://www.aacc.org/-/media/Files/Science-and-Practice/Practice-Guidelines/Pharmacogenetics/)[157]. Where epigenetic effects in patient populations may differ (for instance, DNA methylation may differ for each sex and/or patient age), the assay would need to be validated using a certain number of samples. Synthetic DNA controls, including plasmids, may be utilized in this scenario to check lot-to-lot changes and daily quality assurance. These controls can detect all potential variants. Each pharmacogenetic test should be validated using samples whose genotype has been independently confirmed. The caliber of the supplied nucleic acids determines how well a full genome is amplified. Non-identical allelic representation for the entire genome amplified samples can be caused by reference DNA of poor quality. Therefore, it is necessary to establish the full genome amplified sample's capacity to serve as a control before using it in the test.[159] Testing for proficiency is also necessary to guarantee and enhance the caliber of laboratory analysis. A PGx genotyping test's results may be restricted in their robustness and scope by the analysis's methodology. Therefore, it is risky to link a phenotype to a genotype when the genotype is to be linked to clinical metabolizer status in the absence of scientifically confirmed data. Nevertheless, despite numerous obstacles from the beginning of pharmacogenetics to the present and even in the future, understanding of the subject has significantly improved because of the integration and use of diverse technologies in molecular biology and other supporting science disciplines.[160] Therefore, on the foundation of pharmacogenetic testing, the present, and the future would witness the conversion of discovered gene-drug interactions into therapeutic applications.

Data handling

The use of software logic enables the laboratory to flag potential flawed results to make advantage of If the test yields a patient's genotype combination that is statistically unlikely. By using this technology, the laboratory will be able to repeat the assay before informing the patient of the results, ensuring the validity of the test and their safety. Analytical platforms frequently utilize software to identify anomalous analytical results. It is also possible to manually examine the software calculations periodically.[157]

CONCLUSION

The profiling of SNP variations may replace the "one size fits all" approach to pharmaceutical prescription and enable patients to receive personalized prescriptions that are suited to their individual needs. Additional research in this area may help predict drug responses, improve individual prescribing, maximize treatment efficacy, produce better patient outcomes in the study population, and improve the therapeutic effectiveness and public health. It should also have implications in reducing the risk of side effects and toxicity. However, more research must be done before pharmacogenetics can be included in basic healthcare and prescription writing. Despite the high expectations, certain practical challenges will require focused work in the future, such as legislation and other changes that could influence a patient's unique response to a medicine. Pharmacogenetics (PGx) would therefore continue to play a major part in delivering the promises of personalized medicine: offering a medical treatment customized to the patient's genetic architecture by making progress in the research of

genetic determinants of drug reactions.

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