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Review Article

A REVIEW ON GENETIC POLYMORPHISM OF CYTOCHROME P-450 2C19 AND ITS CLINICAL VALIDITY

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ABSTRACT
Enzymes responsible for the activation and metabolism of drugs and other compounds in humans show wide inter-individual variation in their protein expression or catalytic activity, resulting in unique drug metabolism phenotypes. Pharmacogenetics is the study of the linkage between an individual's genotype and that individual's ability to metabolize a foreign compound. Cytochrome P-450 (CYP) enzyme system is one of the most important drug metabolizing enzyme systems in humans. CYP2C19 isoenzyme metabolizes many clinically important therapeutic agents, notably proto-pump inhibitors, proguanil and benzodiazepines and is mainly found in the Caucasians, Africans, Americans and Oriental populations. Genetic polymorphism plays a vital role in determining the causes of variation in drug metabolism and studies on genetic polymorphism should be carried out by involving the correlation of phenotype-genotype data along with observations that relate them to the findings such as adverse drug reaction monitoring, drug therapy decisions like cost and duration of therapy.

KEYWORDS: Pharmacogenetics, polymorphism, metabolism, phenotyping, metabolites etc.

INTRODUCTION

Individual response to a drug is governed by many factors such as genetics, age, gender, environment and disease. The influence of genetic factors on the response of a drug is a known fact. Study of the influence of genetic factors on drug response and metabolism is termed as Pharmacogenetics. The term "pharmacogenetics" was first coined by Friedrich Vogel in 1959. If the knowledge of pharmacogenetics is applied during drug dosing or drug selection, one can avoid adverse reactions, predict toxicity or therapeutic failure and thus enhance therapeutic efficiency with improvement in clinical outcomes[1].

Pharmacogenetics has elucidated the genetic basis for inter-individual variability in drug response and will continue to play a key role in defining strategies to optimize drug therapy. It is one of the most rapidly growing areas and is becoming increasingly important in clinical pharmacy. The pharmacogenetics of drug-metabolizing enzymes is a prominent focus of this field, because genetic makeup is responsible for a significant portion of drug-induced toxicity; many drugs are metabolized by enzymes that are encoded by polymorphically expressed genes[2].

Polymorphism exhibited by drug-metabolizing enzymes is a well known phenomenon. Nowadays, exploration in the field of pharmacogenetics focuses mainly on the characterization of enzymes responsible for drug biotransformation as well as on describing the various sources of variability in enzyme activity[1]. Pharmacogenetics attempts to identify genetic variations leading to unexpected drug effects, to clarify the underlying molecular mechanism, to evaluate the clinical relevance and to develop appropriate phenotyping and genotyping tests[3].

GENETIC POLYMORPHISM

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles, in which the least common allele has a frequency of about 1% or greater.

The human genome contains approximately 3 billion nucleotide bases, which code for approximately 30,000 genes. Two purine nucleotide bases, adenine (A) and guanine (G), and two pyrimidine nucleotide bases, cytosine (C) and thymidine (T), are present in DNA, with purines and pyrimidines always pairing together as A-T and C-G in the two strands that make up the DNA structure. Most nucleotide base pairs are identical from person to person, with only 0.1% contributing to individual differences. Single-nucleotide polymorphisms, abbreviated as SNPs, are the most common genetic variations in human DNA, occurring approximately once in every 1000 base pairs.

Other examples of genetic variants include:-

- **Insertion-deletion polymorphisms**, in which a nucleotide or nucleotide sequence is either added to or deleted from a DNA sequence.
- **Tandem repeats**, in which a nucleotide sequence repeats in tandem (i.e., if "AG" is the nucleotide repeat unit, "AGAGAGAGAG" is a five-tandem repeat).
- **Frameshift mutation**, in which there is an insertion/deletion polymorphism, and the number of nucleotides added or lost is not a multiple of 3, resulting in disruption of the gene's reading frame.
- **Defective splicing**, in which an internal polypeptide segment is abnormally removed, and the ends of the remaining polypeptide chain are joined.
- **Aberrant splice site**, in which processing of the protein occurs at an alternate site.
- **Premature stop codon polymorphisms**, in which there is premature termination of the polypeptide chain by a stop codon (specific sequence of three nucleotides that do not code for an amino acid but rather specify polypeptide chain termination)[4].

Genetic polymorphism of drug-metabolizing enzymes gives rise to distinct subgroups in the population that differs in their ability to perform certain drug biotransformation reactions. Polymorphism is generated by mutations in the gene for drug-metabolizing enzymes, which causes decreased, increased or absent enzyme activity by multiple molecular mechanisms[1]. Genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism. Extensive metabolism (EM) of a drug is characteristic of the normal population; Poor metabolism (PM) is associated with accumulation of specific drug substrates; and ultra extensive metabolism (UEM) results in increased drug metabolism[3].

Administering an isoenzyme substrate as a probe drug and measuring the metabolite-to-parent drug ratio in plasma and/or urine (metabolic ratio) differentiates extensive metabolizers from poor metabolizers. Poor metabolizers are more likely to have adverse effects from drugs that are substrates of the isoenzyme and decreased efficacy from drugs requiring isoenzyme-mediated activation, while extensive and ultra extensive metabolizers may have therapeutic failure with drugs activated by respective isoenzymes[2].

DRUG METABOLISM

Pharmacokinetics is characterized by four phases:

- Absorption
- Distribution
- Metabolism
- Elimination

Drug Metabolism or biotransformation is defined as "conversion of drug from one chemical form to another". Enzymes involved in drug metabolism are classified into two categories: microsomal and non-microsomal. The microsomal enzymes are derived from rough endoplasmic reticulum and catalyze a majority of drug biotransformation reactions including a number of oxidative, reductive, hydrolytic and glucuronidation reactions. Non-microsomal enzymes are present in soluble form in the cytoplasm and generally attached to the mitochondria. These are also nonspecific enzymes that catalyze few oxidative reactions, a number of reductive and hydrolytic reactions and conjugation reactions other than glucuronidation[3, 5].

Chemical Pathways of Drug Metabolism

R.T.Williams divided the pathways of drug metabolism into two categories: phase I (oxidative) and phase II (conjugative) reactions. These two reaction types often complement each other in function. For example, through catalysis of oxygenation, oxidation, reduction, and hydrolysis reactions, phase I enzymes generate functional groups that may subsequently serve as a site for conjugation to glucuronic acid, sulfate, or glutathione, catalyzed by phase II enzymes[2, 3, 5].

- Phase I reactions:
  - Oxidation
  - Hydroxylation
  - Reduction
  - Hydrolysis
- Phase II reactions:
  - Conjugation (i.e. addition of a new functional group)

Table-1 lists the principal phase I and phase II enzymes found in human liver[3].

CYTOCHROME P-450 ENZYME SYSTEM

Cytochrome P-450 (CYP) enzyme system refers to a group of related enzymes or isoenzymes located in the endoplasmic reticulum and expressed mainly in the liver. It is also present in other organs, such as the intestine, lung, kidney, and brain. This cytochrome P-450 isoenzyme superfamily comprises over 50 heme-containing proteins that catalyze the oxidative metabolism of a large number of structurally diverse drugs and chemicals. It is one of the most important drug-metabolizing-enzyme systems in humans. Cytochrome P-450 exists as multiple forms or isoenzymes, with each having variable distribution in different tissues[1, 2].

A total of 270 CYP-450 gene families found in various organisms have been described till date. There are over 30 human cytochrome P-450 isoenzyme systems that have been identified and the major ones responsible for drug metabolism are the CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and the CYP3A5-7. All CYP isoenzymes in the same family have at least 40% structural similarity, and those in the same subfamily have at least 60% structural similarity[1, 2].

CYP Probe Drugs

Probe substrates (or probe drugs) are compounds that are predominantly or exclusively metabolized in vitro by an individual CYP enzyme. Drugs that are selectively metabolized and that can be safely administered to humans may be used as in vivo probe drugs for the purposes of estimating enzyme activity (i.e., phenotyping) [6].

The phenotyping procedure typically involves the administration of the probe drug (the metabolism of which is known to be solely dependent on the function of a specific drug-metabolizing enzyme) and the collection of blood and/or urine, in order to determine some measure of the enzyme's functional activity, followed by measurement of the metabolic ratio (MR, defined as the ratio of drug dosage or unchanged drug to metabolite measured in serum or urine). Typically, as small a dose of the probe drug as possible is administered so as to avoid or minimize undesirable clinical effects. Selected in-vitro and in-vivo probe drug substrates for specific CYP enzymes have been identified and listed in Table-2[6].

Cytochrome P-450 2C19 (CYP2C19)

CYP2C19 isoenzyme metabolizes several pharmacologically important therapeutic agents. Extensive and poor metabolizers exist for S-mephenytoin, omeprazole and other proton-pump inhibitors, diazepam, propranolol, imipramine, and amitriptyline. The phenotype was determined using mephenytoin as a probe drug because of the significant correlation between formation of the 4-hydroxymephenytoin metabolite and the amount of CYP2C19 in human liver microsomes. Phenotyping of CYP2C19 with mephenytoin is limited because of concerns about the use of the probe drug, low urinary metabolite concentration, and stability of the metabolite in urine. More recently, omeprazole and other substrates have been used in phenotyping studies[2]. Table-3 lists the important pharmaceutical substrate of CYP2C19[3].

CYP2C19 is mainly found in the Caucasians, Africans, Americans and Oriental populations. There are also ethnic differences in the frequency of the poor metabolizer phenotype. About 3-5% of Caucasians and 12- 23% of most Asian populations are poor metabolizers[2].

CLINICAL VALIDITY

Variability in drug response can be attributed in part to variability in the activity of drug–metabolizing enzymes. One of the most important drug metabolizing enzyme systems in humans is the cytochrome P-450 (CYP) enzyme family, which is responsible for the oxidative metabolism of numerous endogenous compounds and xenobiotics. The clinical relevance of factors that influence CYP-mediated metabolism

can be appreciated by estimating in vivo enzyme activity (i.e., the phenotype) through the use of "probe drugs". Thus, the use of probe drugs alone or in combination (i.e., the cocktail approach) can provide an invaluable tool to explore the clinical relevance of genetic and nongenetic factors that affect CYP enzyme activity and thereby contribute substantially to variability in response to therapeutic drugs[6].

CYP2C19 metabolizes many clinically important drugs, notably proto-pump inhibitors, proguanil and benzodiazepines. The clinical implications of the CYP2C19 metabolism are many and varied, including marked efficacy in the treatment of Helicobactor pylori infection with omeprazole in PMs, increased risk of failure of anti-malarial prophylaxis with proguanil and so on[7].

Polymorphism in the CYP2C19 enzymes can lead to excessive or prolonged therapeutic effect or drug-related toxicity after administration of a "typical" dose by failing to clear a drug from the blood or by changing the pattern of metabolism to produce toxic metabolites. This is particularly true of drugs with a narrow therapeutic index. Adjustment of drug dosage could be beneficial based upon knowledge of these differences in metabolism, particularly for individuals possessing the poor and ultrarapid metabolizer phenotypes (because of the variability in the knowledge of clinical utility with specific drugs that are metabolized by the CYP2C19 enzymes, clinicians should use professional judgement in the interpretation and application of results from this phenomenon)[8].

Most of the current literature related to pharmacogenetics of CYP2C19 has studied polymorphisms in Caucasians, Africans, Americans and Oriental populations. Data from the populations of Chinese[9,10], Koreans[11,12], Indians[7,13,14], Mexicans[15,16,17], Turkish[18], Thai[19], Burmese[19], Karen[19], Malaysian[20], Tanzanian[21], Dutch[22], Japanese[23], and few others are well reported and published. Among the Indian population, studies on genetic polymorphism of CYP2C19 are reported for South Indians[14,15], Gujrati and Marwadi Subjects of Mumbai[7] and in few North Indians[24,25,26] as well.

As reported earlier, it was seen that omeprazole metabolic ratio is a safe and convenient means for assessing the in-vivo activity of CYP2C19. The poor metabolizers (PMs) frequency found by phenotyping are belongs to South Indians (14%)[13], Gujratis and Marwadis (10.32%)[7], Southeast Asians like Thai (9.2%), Burmese (11%) and Karen (8.4%)[19], Koreans (12.6%)[12,13], Mexicans (31%)[17] and Japanese (27-35%)[23]. Many factors like age, gender, environment and disease can influence an individual response to a drug and also categorize as the contributing factors for variation in metabolism of drugs like omeprazole and many others.

CONCLUSION

Genetic polymorphism plays a vital role in establishing phenotype status of the study population and also helps in determining the causes of variation in drug metabolism. Studies on genetic polymorphism should be carried out by involving the correlation of phenotype-genotype data along with observations that relate them to the findings such as adverse drug reaction monitoring, drug therapy decisions like cost and duration of therapy. Other focuses like underlying causes of variation in drug metabolism such as age, gender, environment, disease, mutation in gene etc. should also be detailed in further researches in the field of pharmacogenetics.

REFERENCES

1. Abraham BK, Adithan C. Genetic polymorphism of CYP2D6. Indian Journal of Pharmacology 2001; 33: 147-169.
2. Ma MK, Woo MH, Mcleod HL. Genetic Basis of Drug Metabolism. Am J Health-Syst Pharm 2002; 59: 2061-2069.
3. Linder MW, Prough RA, Valdes R. Pharmacogenetics: a laboratory tool for optimizing therapeutic efficiency. Clin Chem 1997; 43: 254-266.
4. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: A Pathophysiologic Approach. 6th Edition, McGraw Hill Companies, pp. 75-90.
5. Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics: A Treatise. Vallabh Prakashan. pp. 111-158.
6. Frye RF. Probing the world of Cytochrome P450 enzymes. Molecular Interventions 2004; 4: 157-162.

7. Gogtay NJ, Panchabhai TS, Noronha SF, Davis S, Shinde VM, Kshirsagar NA. Evaluation of the activity of CYP2C19 in Gujrati and Marwadi subjects living in Mumbai (Bombay). BMC Clinical Pharmacology 2006; 6: 8 (available at: http://www.biomedcentral. com/1472-6904/6/8).
8. Roche Diagnostics, AmpliChip CYP450 test product monograph (available at http://www.amplichip.us/documents/Product_Monograph.pdf).
9. Shu Y, Wang LS, Xu ZH, He N, Xiao WM, Wang W, Huang SL, Zhou HH. 5-Hydroxylation of Omeprazole by Human Liver Microsomal Fractions from Chinese Populations Related to CYP2C19 Gene Dose and Individual Ethnicity. J Pharmacol Exp Ther 2000; 295: 844-851.
10. Andersson T, Regardh CG, Lou YC, Zhang Y, Dahl ML, Bertilsson L. Polymorphic hydroxylation of S-mephenytoin and omeprazole metabolism in Caucasian and Chinese subjects. Pharmacogenetics 1992; 2: 25-31.
11. Lee J, Yoon Y, Jeong H, Lee S, Liu K, Kang W, Cha I, Shin J. Extensive evaluation of genetic polymorphism of CYP2C19 in a Korean population: interethnic comparison among Asian populations. Clin Pharmacol Ther 2004; 75: 69.
12. Roh HK, Dahl ML, Tybring G, Yamada H, Cha YN, Bertilsson L. CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. Pharmacogenetics 1996; 6: 547-551.
13. Rosemary J, Adithan C, Padmaja N, Shashindran CH, Gerard N, Krishnamoorthy R. The effect of the CYP2C19 genotype on the hydroxylation index of omeprazole in South Indians. Eur J Clin Pharmacol 2005; 61: 19-23.
14. Adithan C, Gerard N, Vasu S, Rosemary J, Shashindran CH, Krishnamoorthy R. Allele and genotype frequency of CYP2C19 in a Tamilian population. Br J Clin Pharmacol 2003; 56: 331-333.
15. Gonzalez HM, Pomero TEM, Peregrina LAA, Chavez CTJ, Escobar-Islas E, Lozano KF, Hoyo-Vadillo C. CYP2C19- and CYP3A4-Dependent Omeprazole Metabolism in West Mexicans. J Clin Pharmacol 2003; 43; 1211-1215.
16. Luo HR, Poland RE, Lin KM, Wan YJY. Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: A cross-ethnic comparative study. Clin Pharmacol Ther 2006; 80: 33-40.
17. Gonzalez HM, Gomez-Martinez LE, Chavez TC, Trevino R, Romero EM, Hoyo-Vadillo C. Prevalence of CYP2C19 and CYP3A4 in Poor Metabolizers among Inhabitants of Tlaxcala, Mexico. Proc West Pharmacol Soc 2006; 49: 102-103.
18. Kortunay S, Basci NE, Bozkurt A, Isimer A, Sayal A, Kayaalp SO. The hydroxylation of omeprazole correlates with S-mephenytoin and proguanil metabolism. Eur J Clin Pharmacol 1997; 53: 261-264.
19. Tassaneeyakul W, Mahatthanatrakul W, Niwatananun K, Bangchang KN, Tawalee A, Krikreangsak N, Cykleng U, Tassaneeyakul W. CYP2C19 Genetic Polymorphism in Thai, Burmese and Karen Populations. Drug Metabolism and Pharmacokinetics 2006; 21: 286-290.
20. Yang YS, Wong LP, Lee TC, Mustafa AM, Mohamed Z, Lang CC. Genetic polymorphism of cytochrome P450 2C19 in healthy Malaysian subjects. Br J Clin Pharmacol 2004; 58: 332–335.
21. Bathum L, Skjelbo E, Mutabingwa TK, Madsen H, Horder M, Brosen K. Phenotypes and genotypes for CYP2D6 and CYP2C19 in a black Tanzanian population. Br J Clin Pharmacol 1999; 48: 395-401.
22. Tamminga WJ, Wemer J, Oosterhuis B, Wieling J, Wilffert B, Leij LFMH, Zeeuw RA, Jonkman JHG. CYP2D6 AND CYP2C19 Activity in a large population of Dutch healthy volunteers: indications for oral contraceptive-related gender differences. Eur J Clin Pharmacol 1999; 55: 177-184.
23. Kimura M, Ieiri I, Wada Y, Mamiya K, Urae A, Iimori E, Sakai T, Otsubo K, Higuchi S. Reliability of the omeprazole hydroxylation index for CYP2C19 phenotyping: possible effect of age, liver disease and length of therapy. Br J Clin Pharmacol 1999; 47: 115-119.
24. Lamba JK, Dhiman RK, Singh R, Kohli KK. Correlation between omeprazole hydroxylase and CYP2C19 genotype in North Indians. Eur J Clin Pharmacol 2001; 57: 649-652.
25. Lamba JK, Dhiman RK, Kohli KK. CYP2C19 genetic mutations in North Indians. Clin Pharmacol Ther 2000; 68: 328-335.
26. Lamba JK, Dhiman RK, Kohli KK. Genetic polymorphism of the hepatic cytochrome P450 2C19 in North Indian subjects. Clin Pharmacol Ther 1998; 63: 422-427.

Table 1: Principle Phase I and Phase II Enzymes in Human Liver

Phase I	Phase II
CYP1A2 CYP2A6 CYP2B CYP2C9* CYP2C19* CYP2D6* CYP2E1* CYP3A NADPH-quinone Oxido-reductase*	Glutathione S-transferase* N-acetyltransferase* UDP-glucurosyltransferase* Sulfotransferases

* Enzymes known to exhibit polymorphism in humans

Table 2: Selected *In Vitro* and *In Vivo* Probe Substrates for CYP Enzymes

CYP	Probe Substrate	
	In Vitro	***In Vivo***
CYP1A2	Phenacetin	Caffeine
CYP2A6	Coumarin	Courmarin
CYP2B6	Bupropion	Bupropion
CYP2C8	Paclitaxel	Unclear
CYP2C9	Diclofenac Tolbutamide S-Warfarin	Tolbutamide S-Warfarin
CYP2C19	S-Mephenytoin Omeprazole	S-Mephenytoin Omeprazole
CYP2D6	Bufurolol	Dextromethrophan
CYP2E1	Chlorzoxazone	Chlorzoxazone
CYP3A4	Midazolam Testosterone	Midazolam Erythromycin

Table 3: Pharmaceutical substrate of CYP2C19

• Amitriptyline • Clomipramine • Certain barbiturates • Omeprazole • Citalopram • Chlorproguanil	• Imipramine • Propranolol • Mephenytoin • Proguanil • Diazepam