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Research Article

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Pharmacogenetics of Drug Metabolizing Enzymes

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Declaration

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Abstract

The pharmacogenetics of drug metabolising enzymes in the context of effect of single nucleotide polymorphisms on clinically significant changes in enzymatic activities/expression is discussed. Genetic polymorphisms explain partly the inter-individual variability in drug induced adverse reactions, toxicity and therapeutic responses. Several SNPs which are extensively studied for pharmacogenetic aspects are present in genes encoding for glucose-6-phosphate dehydrogenase, N-acetyltransferase and the superfamily of cytochrome P-450 (CYP) isoenzymes. Drug therapy will be optimised by pharmacogenetic studies assessing the role of genetic markers in drug responsiveness and toxicity. The application of pharmacogenetics will eventually increase the efficacy of drugs with decrease in risk of adverse events due to over- and under-dosing.

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Introduction

Pharmacogenetics is the "study of the role of genetics in drug response" as defined by Friedrich Vogel. In the era of personalized medicine, pharmacogenetics based treatment of patients is a much studied area and it is becoming the need of time to reduce the risks associated with the use of drugs in clinical practice. Pharmacogenetic of drug metabolizing enzymes is a very important and interesting focus of this field. Variations in the genetic makeup of patients result in different dosing requirements as many of the drugs are metabolized by enzymes that are encoded by polymorphic genes [1]. Drug induced toxicity can be prevented if patient's pharmacogenetic makeup is considered before dosing a particular drug in a specific amount. Genetic changes in specific metabolic pathways, producing clinically significant phenotypes, can be identified by genotype analysis. Based on this information, patients can be classified as poor, intermediate or extensive metabolizers of certain drugs. This classification is based on patient's ability to metabolize particular drug and it can differentiate inter- and intra-patient pharmacokinetic and pharmacodynamics variability [2-4]. It is not necessary that all the genetic polymorphisms in the genes coding drug metabolizing enzymes are clinically relevant. The clinical significance of a genetic change increases if there is wide usage of the drug with narrow therapeutic range, drug is principally cleared by the enzyme pathway, or there are limited therapeutic alternatives available. Pharmacogenetic evidences have shown that interindividual differences in drug related toxicity and therapeutic response are not peculiar to a particular individual or group. The use of pharmacogenetics information in clinical settings for daily patient care has the potential to guide a safe and effective therapy. However, there is a need to collect more evidences to develop pharmacogenetics applications that can be used in clinical settings. Based on these findings, patient care in future can be benefitted by pharmacogenetic applications.

Drug metabolism occurs by biochemical modification of pharmaceutical molecules by specialised enzymatic systems in living organisms. There are three phases of drug metabolism, namely phase I, phase II and phase III. In phase I, reactive and polar groups are added to the substrate molecules by the processes of oxidation, reduction or hydrolysis. In phase II, the activated substrates are conjugated with charged species on carboxyl, hydroxyl, amino or sulfhydryl groups. In the phase III of drug metabolism conjugated metabolites are further processed before being excreted out of the cells. Many studies have been done on the pharmacogenetics differences in a number of phase-I enzymes, such as cytochrome P-450 (CYP) isoenzymes, dehydrogenases, and esterases. Phase-II (conjugating) enzymes have also been looked for pharmacogenetics differences. The present review discusses the pharmacogenetics aspects of drug metabolising enzymes in the light of clinically significant single nucleotide polymorphisms (SNPs) in the DNA coding for drug metabolising enzymes. Observation of the adverse reactions (phenotypes) in patients, receiving common doses of drugs, has led to the discoveries of a number of pharmacogenetics variants (genotypes). The drug metabolizing enzymes are primarily located in liver and, to some extent, in other organs, like small intestine.

Glucose-6-phosphate dehydrogenase

In early 1950s, antimalarial drugs were found to cause haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. These were the first observations of the phenotypic variations in individual's responses towards certain

drugs. G6PD is expressed in all tissues of the body and controls the flow of carbon through the pentose phosphate pathway. NADPH is produced by G6PD for reductive biosynthesis. G6PD balances the oxidation-reduction in the cell and glutathione is kept in a reduced state [5, 6]. In G6PD deficiency, reduced glutathione is absent and allows oxidative drugs to oxidize sulfahydroxyl groups of haemoglobin, causing haemolysis. In G6PD deficient patients, haemolytic anaemia is caused by drugs like primaquine, sulfones, sulfonamides, nitrofurans, vitamin K analogues, cefotetan and chloramphenicol. There are reports of 30 different mutations in the G6PD gene in its exonic regions [7, 8]. Most of these mutations are C>G changes and only single is not a point mutation [9]. These mutations result in lower G6PD activity causing lower glutathione concentrations in erythrocytes. These conditions cause haemolytic anaemia following the ingestion of certain drugs [10]. Different ethnic groups don't have similar prevalence of G6PD deficiency. For example, G6PD deficiency is more commonly observed phenomenon in African and Mediterranean males. Africans show two mutations, G6PD A and G6PD A(-). Normal red cell activity is associated with G6PD A mutation. The G6PD A(-) protein is unstable in vivo and produces only about 10% of the normal activity. G6PD A is an adenosine-to-guanine substitution at nucleotide 376 (A376G) which replaces an asparagine residue by aspartic acid residue [11]. In case of G6PD A(-) mutation, 3 different variants exist in one allele. In Mediterraneans, C563T is the most commonly observed mutation resulting in an amino acid change (Ser188Phe). The use of cyclosporine, tacrolimus, penicillin, and cefotetan is also shown to cause drug-induced haemolytic anaemia [12]. There are many confounding factors, such as, dose, duration of therapy, and other oxidant stresses associated with the risk and severity of haemolysis. Genotyping of patients for G6PD deficiency is not advocated due to these factors. The toxicity is rare, not life threatening and there is poor predictive ability of genotype about the development of haemolytic anaemia. G6PD deficiency provides an example of the development of genotypic analysis much after clinical observation and no added clinical advantages of genotyping.

N-Acetyltransferase

N-Acetyltransferase is a phase II conjugating liver enzyme which catalyses the N-acetylation and O-acetylation of arylamine, carcinogens and heterocyclic amines. A SNP in the gene coding this enzyme is one of the earlier studied genotype-phenotype relation in the field of pharmacogenetics. It has been observed that the slow acetylation by this enzyme results in toxic conditions in patients receiving drugs like isoniazid, sulfonamides, procainamide, and hydralazine. The fast acetylator phenotype causes difficulties in terms of non-responsiveness towards isoniazid and hydralazine in treatment of tuberculosis and hypertension respectively. The differences in the acetylation of substrate drug molecules are explained by the allelic variations at the NAT2 gene locus. NAT2 gene consists of open-reading frames (i.e. protein coding regions) with no introns and has 27 alleles reported. Two or three point mutations are present in most NAT2 variant alleles. NAT2 variants are most studied for their association with increased risk for cancers. There is prolonged exposure of cells to carcinogens in slow acetylators as compared with fast acetylators [13]. In a small Japanese population, there is association between impaired isoniazid metabolism and NAT2 mutations [14]. Genotyping of NAT2 mutations may provide an aid to traditional therapeutics involving isoniazid prescription in future.

Cytochrome P450 isoenzymes

Genetic polymorphisms in the Cytochrome P450 isoenzymes present another example of pharmacogenetics of drug metabolizing enzymes. There are more than 50 heme-containing proteins catalysing the oxidative metabolism of many drugs and chemical compounds. This superfamily of enzymes is one of the most studied drug metabolizing enzymes. The name CYP 450 is derived from the fact that the CYP 450 enzyme has a characteristic maximum absorbance at 450 nm in its reduced form. Multiple forms or isoenzymes exist for the CYP enzyme, each of which is variably distributed in different tissues of the body. The nomenclature of the CYP 450 enzymes has been done on the basis of the similarity in the genetic sequences that code for the isoenzymes [15]. The rule for two isoenzymes to be in different CYP families is set as less than 40% similarity to each other. CYP nomenclature can be found in detail in literature [16, 17].

The metabolism of 25-30% of all clinically used medications, including dextromethorphan, β-blockers (e.g., metoprolol), antiarrhythmics, antidepressants (e.g., fluvoxamine, fluoxetine, imipramine, nortriptyline), antipsychotics (e.g., haloperidol, risperidone), morphine derivatives, and many other drugs is done by CYP2D6 isoenzymes. Genetic polymorphisms in CYP2D6 gene are responsible for the variability in the interindividual responses to these drugs. Amongst all of the CYP isoenzymes, CYP2D6 gene has the highest number of variations. The mutations in the CYP2D6 gene result in a reduction or complete loss of activity [18]. Extensive metabolisers are differentiated from poor metabolisers by administrating a CYP2D6 substrate as a probe drug and taking measurement of metabolic ratio (metabolite-to-parent drug ratio) in the urine. Two non-functional allele are present in poor metabolisers as shown by genotype-phenotype studies [19, 20]. Gene duplication of CYP2D6 gene results in an ultrarapid metaboliser phenotype [21]. The clearance of fluoxetine, fluvoxamine, desipramine and mexiletine is predicted by CYP2D6 genotyping [22]. The adverse effects of antidepressants and neuroleptics can also be predicted by CYP2D6 genotyping. As an example of use of pharmacogenetics in clinical practice, dosage recommendations of antidepressants based on CYP2D6 genotypes are available [23]. However, there is a need to do prospective studies to judge the positive outcomes of genotype based therapy.

Phenytoin, S-warfarin, tolbutamide, losartan and NSAIDs are metabolized by CYP2C9 isoenzyme and there are evidences of impaired metabolism of these drugs [24]. There are three allelic variants of CYP2C9 gene resulting in the decreased enzymatic activity of the enzyme and so its metabolic activity [25]. CYP2C9*2 (Arg144Cys) and *3 (Ile359Leu) are the variant alleles that result in single amino acid substitutions. Compared with the wild type allele, CYP2C9*2 and *3 are associated with ~5 and ~27 fold decrease in the intrinsic clearance of S-warfarin respectively [26]. The clinical impact of CYP2C9*3 polymorphism is more severe than the *2 polymorphism. These polymorphisms are particularly important in patients receiving vitamin K antagonists as oral anticoagulants as there are increased risks of bleeding were in patients with mutant alleles (poor metabolizers). Such patients require frequent monitoring of anticoagulation response and dosage adjustments [27]. Phenytoin is principally metabolised by CYP2C9 and to some extent by CYP2C19 isoenzymes. Therefore, mutations in the CYP2C9 gene have greater impact on phenytoin toxicity. The risk of adverse drug reactions due to phenytoin or warfarin toxicity can be reduced by genotyping of CYP2C9 variants [27].

CYP2C19 is another member of the CYP 450 superfamily of drug metabolizing enzymes which breaks down many pharmacologically important therapeutic drugs. Two non-functional alleles result in the poor metaboliser phenotype of CYP2C19. The heterozygous and homozygous dominant genotypes result in extensive metaboliser phenotype. The heterozygous and homozygous dominant genotypes cannot be distinguished by phenotyping methods. There are five mutations identified in CYP2C19 gene [28]. CYP2C19*2 and *3 are the most common variant alleles responsible for the poor metaboliser phenotype. These variants arise from single base pair substitutions in exons 4 and 5 respectively. The single base pair substitutions introduce premature stop codons and result in deformation of polypeptide chains with loss of functionality [29]. CYP2C19 genotyping is important in predicting the inter-individual differences in plasma concentrations of proton pump inhibitors. Another example of a substrate metabolised by CYP2C19 is Diazapam and its metabolism is affected by the CYP2C19*2 polymorphism. In individuals where Diazapam is slowly metabolised, there is increased risk of over dosing and toxicity.

CYP3A subfamily is one of the most important classes of CYP enzymes. CYP3A isoenzymes are primarily expressed in liver and small intestine [30]. About half of the currently used drugs are metabolised by hepatic CYP3A4 whereas intestinal CYP3A4 isoenzyme metabolises most of the orally administered drugs [31]. Variations in the genetic expression of CYP3A4 affect the metabolism of its substrates (HIV protease inhibitors, benzodiazepines, calcium channel blockers, hydroxymethylglutaryl coenzyme A- reductase inhibitors, antineoplastic drugs, nonsedating antihistamines and immunosuppressants). Drug efficacy and toxicity also differ in individuals due to these variations. There are three isoenzymes CYP3A4, CYP3A5 and CYP3A7 that sum up to the activity of CYP3A4 [32]. CYP3A4 activity can vary by 50 folds between individuals. CYP3A5 is an important contributor in the inter-individual and interracial differences in the metabolism of CYP3A substrates. Patients with wild status for CYP3A4 and CYP3A5 genotypes are at increased risk of toxicity and show lack of therapeutic effect due to extensive metabolism of drugs. CYP3A pharmacogenetics can be used to provide guidelines to modulate drug therapy in future.

Summary

Identification of SNPs is becoming easier through advancements in high-throughput technological inventions. The huge information databases of the genetic changes are expanding and easily accessible. Pharmacogenetics explains the toxicity and inefficacy in some patients. Pharmacogenetics continues to develop as a promising area towards personalised therapeutic approach. In future, it may become possible that patient's response and need of drug doses be prospectively decided based on their genetic backgrounds. This is particularly important in case of drugs with narrow therapeutic window where there are risks of over- and under-dosing. A person's ability to metabolise certain drugs can be judged more accurately based on genotyping than based solely on ethnicity. Pharmacogenetics of drug metabolism can help in learning how to administer certain drugs safely and effectively. With enhancements in genotyping techniques in terms of cost to benefit ratio, pharmacogenetics may lead to individualisation of medication.

Conclusion

Pharmacogenetics of drug metabolising enzymes has explained the genetics behind inter-individual differences in drug responses. Its application in clinical settings can help to tailor safe and effective pharmacotherapy for individual patients.

Refrences

1. Phillips, K.A., et al., *Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review.* JAMA, 2001. **286**(18): p. 2270-9.
2. Evans, D.A., et al., *Interethnic variability in human drug responses.* Drug Metab Dispos, 2001. **29**(4 Pt 2): p. 606-10.
3. Kalow, W., et al., *Ethnic difference in drug metabolism: debrisoquine 4-hydroxylation in Caucasians and Orientals.* Can J Physiol Pharmacol, 1980. **58**(9): p. 1142-4.
4. Wood, A.J., *Ethnic differences in drug disposition and response.* Ther Drug Monit, 1998. **20**(5): p. 525-6.
5. Kletzien, R.F., P.K. Harris, and L.A. Foellmi, *Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress.* FASEB J, 1994. **8**(2): p. 174-81.
6. Beutler, E., *G6PD deficiency.* Blood, 1994. **84**(11): p. 3613-36.
7. Vulliamy, T.J., et al., *Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anemia.* Proc Natl Acad Sci U S A, 1988. **85**(14): p. 5171-5.
8. Beutler, E., *The molecular biology of G6PD variants and other red cell enzyme defects.* Annu Rev Med, 1992. **43**: p. 47-59.
9. Vulliamy, T., P. Mason, and L. Luzzatto, *The molecular basis of glucose-6-phosphate dehydrogenase deficiency.* Trends Genet, 1992. **8**(4): p. 138-43.
10. Carson, P.E., et al., *Electrophoretic demonstration of stromal effects on haemolysate glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase.* Nature, 1966. **210**(5038): p. 813-5.
11. Hirono, A. and E. Beutler, *Molecular cloning and nucleotide sequence of cDNA for human glucose-6-phosphate dehydrogenase variant A(-).* Proc Natl Acad Sci U S A, 1988. **85**(11): p. 3951-4.
12. Stroncek, D., J.L. Procter, and J. Johnson, *Drug-induced hemolysis: cefotetan-dependent hemolytic anemia mimicking an acute intravascular immune transfusion reaction.* Am J Hematol, 2000. **64**(1): p. 67-70.
13. Grant, D.M., et al., *Pharmacogenetics of the human arylamine N-acetyltransferases.* Pharmacology, 2000. **61**(3): p. 204-11.
14. Kita, T., et al., *N-Acetyltransferase2 genotype correlated with isoniazid acetylation in Japanese tuberculous patients.* Biol Pharm Bull, 2001. **24**(5): p. 544-9.
15. Nelson, D.R., et al., *P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature.* Pharmacogenetics, 1996. **6**(1): p. 1-42.
16. Ingelman-Sundberg, M., et al., *Human cytochrome P-450 (CYP) genes: a web page for the nomenclature of alleles.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(12): p. 1307-8.
17. Ingelman-Sundberg, M., et al., *Human cytochrome P450 (CYP) genes: recommendations for the nomenclature of alleles.* Pharmacogenetics, 2000. **10**(1): p. 91-3.
18. Ingelman-Sundberg, M. and W.E. Evans, *Unravelling the functional genomics of the human CYP2D6*

gene locus. Pharmacogenetics, 2001. **11**(7): p. 553-4.
19. Johansson, I., et al., *Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation.* Mol Pharmacol, 1994. **46**(3): p. 452-9.
20. Kagimoto, M., et al., *Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes.* J Biol Chem, 1990. **265**(28): p. 17209-14.
21. Kroemer, H.K. and M. Eichelbaum, *"It's the genes, stupid". Molecular bases and clinical consequences of genetic cytochrome P450 2D6 polymorphism.* Life Sci, 1995. **56**(26): p. 2285-98.
22. Hamelin, B.A., et al., *The disposition of fluoxetine but not sertraline is altered in poor metabolizers of debrisoquin.* Clin Pharmacol Ther, 1996. **60**(5): p. 512-21.
23. Kirchheiner, J., et al., *CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages.* Acta Psychiatr Scand, 2001. **104**(3): p. 173-92.
24. Bhasker, C.R., et al., *Allelic and functional variability of cytochrome P4502C9.* Pharmacogenetics, 1997. **7**(1): p. 51-8.
25. Sullivan-Klose, T.H., et al., *The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism.* Pharmacogenetics, 1996. **6**(4): p. 341-9.
26. Haining, R.L., et al., *Allelic variants of human cytochrome P450 2C9: baculovirus-mediated expression, purification, structural characterization, substrate stereoselectivity, and prochiral selectivity of the wild-type and I359L mutant forms.* Arch Biochem Biophys, 1996. **333**(2): p. 447-58.
27. Higashi, M.K., et al., *Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy.* JAMA, 2002. **287**(13): p. 1690-8.
28. Ibeanu, G.C., et al., *Identification of new human CYP2C19 alleles (CYP2C19*6 and CYP2C19*2B) in a Caucasian poor metabolizer of mephenytoin.* J Pharmacol Exp Ther, 1998. **286**(3): p. 1490-5.
29. Goldstein, J.A. and S.M. de Morais, *Biochemistry and molecular biology of the human CYP2C subfamily.* Pharmacogenetics, 1994. **4**(6): p. 285-99.
30. Cholerton, S., A.K. Daly, and J.R. Idle, *The role of individual human cytochromes P450 in drug metabolism and clinical response.* Trends Pharmacol Sci, 1992. **13**(12): p. 434-9.
31. Paine, M.F., et al., *First-pass metabolism of midazolam by the human intestine.* Clin Pharmacol Ther, 1996. **60**(1): p. 14-24.
32. Kolars, J.C., et al., *CYP3A gene expression in human gut epithelium.* Pharmacogenetics, 1994. **4**(5): p. 247-59.