# Role of phenotyping and genotyping in the measurement of variability in human drug response

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# ABSTRACT

Among the causes of variability in human drug response, interindividual variation in enzymes, receptors and transporters play an important role. Best known are the interindividual differences in drug metabolism, caused by genetic-environmental interactions, especially for those drugs mainly metabolised by genetically polymorphic enzymes. The population was divided in extensive (EM) and slow, or so called poor metabolisers (PM). Side effects with normal doses were to be expected in PM patients treated with drugs metabolised by the polymorphic enzymes and perhaps lack of therapeutic effect in the faster EMs. Several drugs suffering polymorphic metabolism were withdrawn and the pharmaceutical industry usually decided not to develop drugs suffering polymorphic metabolism. However, most of the drugs metabolised in the body suffer interindividual differences in metabolism but that does not necessarily mean that clinically significant problems are always to be expected. On the other hand, the fast development of pheno and genotyping methodologies make it feasible to predict kinetics, and in many cases drug response, early in drug development and therefore prevent clinical problems in healthy volunteers or patients. The recent mibefradil case in the US could be an interesting example for consequences of not considering this issue at the right moment.

Key words: Pharmacogenetics, genetic polymorphisms, enzymes, drug effects.

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### INTRODUCTION

Among the causes of variability in human drug response interindividual variation in human genes coding for enzymes, receptors and transporters could play an important role. Besides, dynamic interactions are known to occur between genetic and environmental factors causing large interindividual variations in drug response. Since the discovery of genetic polymorphism in the metabolism of isoniazid in the early 60s (now known to be mediated by N-acetyl transferase 2 or NAT2) and even more after a similar discovery for debrisoquine in the 70s

(CYP2D6), and other cytochrome isoenzymes thereafter, some of the causes for interindividual differences in drug response were identified. As it often happens, too simplistic conclusions were reached at the beginning. So the population was divided in extensive (EM) and slow or so called poor metabolisers (PM) and side effects with normal doses were to be expected in PM patients treated with drugs metabolised by the polymorphic enzymes. Likewise, lack of therapeutic effect in the faster EM was expected. Several drugs suffering polymorphic metabolism were withdrawn and the pharmaceutical industry was well advised not to develop drugs suffering polymorphic metabolism. However, most of the drugs metabolised in the body suffer interindividual differences in metabolism but that does not necessarily mean that clinically significant problems are to be expected in all of them. Those drugs, which have important active metabolites and/or several equally important metabolic pathways, mediated by different enzymes and/or have a high therapeutic index, will very rarely cause clinically significant problems because of differences in metabolic capacity.

Recent availability of pheno and genotyping methodology is making possible to prevent or diminish many of these problems, both in drug development and in actual clinical practice. *In vitro* studies are now carried out for most new drugs under development to find out its main metabolic pathways and the enzymes involved. This way some side effects and drug interactions with potential clinical significance can be predicted. Rapid development of newer methodologies, such as DNA sequencing, will make feasible in the near future to extend this knowledge to most drugs metabolised in the body. However, correlation between geno and phenotype and actual drug effects should not be forgotten if we really want to get the best safety and efficacy in treatment to most patients [Kalow, 1989].

### ENZYMES POTENTIALLY IMPORTANT IN DRUG METABOLISM IN MAN

In table 1 main liver enzymes known to be involved in drug metabolism in man are shown. More will appear in the near future but we will concentrate on those already studied in man. All the enzymes shown belong to the cytochrome P-450 system, with the exception of NAT2. The arylamine N-acetyltransferase (NAT-2) polymorphism causes impaired drug metabolism in about half of the white population [Agúndez, 1994],[Agúndez, 1996],[Carrillo, 1994]. However, most of the drugs so far known to be metabolised by this enzyme have either a high therapeutic index, or they are not so commonly used. Therefore, NAT2 has had more implications from the toxicological point of view and for carcinogenic mechanisms [Agúndez, 1995],[Agúndez, 1996],[Agúndez, 1996],[Martínez, 1995]. Drug-metabolizing enzymes encoded by the cytochrome P450 genes, are noted for their high degree of interspecies and intraspecies variability [González, 1990]. Cytochrome P-450 comprises several enzymes but we will consider here only those so far found to be potentially important in for drug metabolism in man.

#### CYP1A2

Phenotyping methods for this enzyme have been developed and tested in different populations by the group of Kalow [Kalow, 1991], [Tang, 1994], [Tang, 1996] and several others [Fuhr, 1996]. Caffeine is the probe more frequently used for CYP1A2 phenotyping [Carrillo, 1996]. The metabolic profile of caffeine biotransformation by CYP1A2 averages

around 80% for paraxanthine, 11% for theobromine and 5% for theophylline formation [Gu, 1992]. So far, no genotyping method is available for CYP1A2 and it is an enzyme with very important environmental influence. Caffeine, smoking and several drugs can modify significantly CYP1A2 phenotype. The atypical antipsychotic drug clozapine, as well as many other psychotropic drugs are metabolised by CYP1A2 to a major extent [Bertilson, 1994]. Moreover, changes in the habitual caffeine intake alter the metabolism of clozapine in schizophrenic patients [Carrillo, 1998].

Table 1.       Main liver enzymes known to be involved in drug metabolism in man			
Enzyme	Usual probe	Genetic polymorphism	Typing methods available
CYP1A2	Caffeine	?	Phenotype
CYP2C9	Diclofenac	Yes	Pheno/Genotype
CYP2C18	Tolbutamide	Yes	Pheno/Genotype
CYP2C19	S-mephenytoin	n Yes	Pheno/Genotype
CYP2D6	Debrisoquine	Yes	Pheno/Genotype
CYP2E1	Chlorzoxazone	e Yes	Pheno/Genotype
CYP3A4	Midazolam	?	Phenotype
NAT2	Caffeine	Yes	Pheno/Genotype

# CYP2C

Four members of this subfamily have been identified so far in humans: CYP2C8, CYP2C9, CYP2C18, and CYP2C19. CYP2C9 and CYP2C19 are much better characterised than CYP2C8 and CYP2C18 in man. CYP2C9 is important in the metabolism of certain therapeutically used drugs including the anticoagulant drug warfarin and a number of nonsteroidal antiinflammatory drugs. A number of allelic variants of CYP2C9 exist in humans, but the effects of these allelic variants on metabolism *in vivo* remain to be determined. Diclofenac is being used as a probe for CYP2C9 phenotyping, although some discrepancies have been found between geno and phenotype [Klose, 1998], [Yamazaki, 1998].

A well-characterised genetic polymorphism occurs in CYP2C19, which is associated with the metabolism of the anticonvulsant drug mephenytoin. In population studies, individuals can be segregated into extensive and poor metabolizers of mephenytoin. Poor metabolizers are unable to 4'- hydroxylate the S-enantiomer of mephenytoin. There are marked interethnic variations in the frequency of the poor metabolizer phenotype, which represents 1 to 5% of Caucasians. Mephenytoin is the most frequently used probe for CYP2C19 phenotyping [Goldstein, 1994],[Stubbins, 1996].

### CYP2D6

The debrisoquine hydroxylase (CYP2D6) catalyzes the oxidative metabolism of more than 40 different clinically important drugs. The CYP2D6 gene is highly polymorphic. Defect alleles, causing the poor metabolizer phenotype, and also alleles with duplicated or

multiduplicated active genes, causing ultrarapid metabolism, have been described. It is clearly the most thoroughly studied drug metabolic polymorphism so far. From 5% to 10% of Caucasians designated as poor metabolizers (PMs) of the debrisoquine/sparteine polymorphism have a severely impaired capacity to metabolize several therapeutically used drugs [Alván, 1990],[Benítez, 1988]. Debrisoquine [Lennard, 1990], followed by dextromethorphan [Henthorn, 1989] and sparteine [Eichelbaum, 1979] are the most frequently used probes for CYP2D6 phenotyping. The gene controlling CYP2D6 is located on the long arm of chromosome 22 [Gaedigk, 1991],[González, 1991],[Linder, 1997]. Ultrarapid metabolism of debrisoquine has been shown in 1% to 30% in several populations [Johansson, 1993],[Akullu, 1996],[Agúndez, 1995]. Several methods for genotyping have been developed [Dahl, 1995],[Johansson, 1996],[Agúndez, 1994]

#### CYP2E1

Ethanol-inducible CYP2E1 is an enzyme of interest because it metabolizes several procarcinogens and solvents to reactive metabolites. CYP2E1 has also been implicated in alcohol liver disease because of its contribution to oxidative stress. However, not many drugs have been found to be metabolized by CYP2E1 so far [Tateishi, 1997]. Chlorzoxazone is being used for CYP2E1 phenotyping [Bachmann, 1996],[Lucas, 1996],[Burckart, 1998]. Previously, polymorphic alleles with mutations in introns and in the 5'-flanking regulatory region have been described, and their presence has been related to the incidence of alcohol liver disease and lung cancer. Genotyping studies in man have shown that the CYP2E1 gene is functionally surprisingly well conserved compared with other cytochrome P450 enzymes active in drug metabolism, which suggests an important endogenous function in humans [Hu, 1997].

# СҮРЗА

CYP3A isoforms are responsible for the biotransformation of a wide variety of exogenous chemicals and endogenous steroids in human tissues. Two members of the CYP3A subfamily display developmentally regulated expression in the liver; CYP3A7 is expressed in the fetal liver, whereas CYP3A4 is the major cytochrome P-450 isoform present in the adult liver. CYP3A4 expression is transcriptionally activated during the first week after birth and is accompanied by a simultaneous decrease of CYP3A7 expression, in such a way that the overall CYP3A protein content remains nearly constant [Lacroix, 1997].

Cytochrome P-450 enzymes of the CYP3A gene subfamily account for up to 30% of the total cytochrome P-450 present in the adult human liver and for the majority of cytochrome P-450 in the human small bowel [Shimada, 1994]. Many *in vitro* and *in vivo* drug interactions have been reported at the CYP3A site. There are pronounced patient differences caused by genetic and environmental factors in the metabolism of CYP3A substrates. Midazolam has been suggested as probe for CYP3A4 phenotyping [Carrillo, 1998]. CYP3A isoforms are also expressed outside the liver and this could have implications, both for the initiation of pulmonary carcinogenesis by agents that require metabolic activation, such as tobacco-derived polycyclic aromatic hydrocarbons and perhaps for local metabolism of topical drugs. CYP3A5 is the predominant CYP3A form in human lung and CYP3A4 is expressed in about 20% of individuals, but considerable variation of pulmonary expression occurs in both CYPs between individuals [Anttila, 1997].

#### CONCLUSIONS

Several enzymes of the cytochrome P-450 system have been found to be causing clinically significant variability in human drug response. Among them CYP1A2, CYP2C9, CYP2D6 and CYP3A4 are perhaps the most important. Pheno and/or genotyping all the possibly involved relevant enzymes should be a must for phase I clinical trials and very useful in certain cases in phase II and III clinical trials for new drugs under development. More basic research in man with regard to genetic-environmental interactions in drug metabolism, as well as that related to drug receptors and transporters, is urgently needed. This will be the only way to avoid toxicity and increase efficacy during drug development as well as to facilitate molecule selection. Although methods for phenotyping and genotyping exist for most relevant enzymes, better possibilities are foreseen in the near future that will make it possible to predict with more accuracy drug metabolism and response.

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# <u>Discussion</u>: Role of phenotyping and genotyping in the measurement of variability in human drug response

"Due to problems with the recording of this presentation, reproduction of the discussion was not possible. The Esteve Foundation apologises to the author and discussants."

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