The impact of genetic polymorphisms in risk assessment of drugs

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Genetic variation is a fundamental characteristic of all living organisms. In humans marked individual variability has been demonstrated at every level of investigation. With regard to drug metabolism variability in responsiveness to a given dose originates from many factors, e.g. environmental influences, alteration of absorption, distribution, excretion and metabolism.

The most important drug metabolizing enzymes are by tradition categorized into two groups, phase I and phase II, according to the chemical reactions they catalyze. Phase I biotransformations include oxygenations, oxidations, reductions and hydrolyses while phase II reactions (sometimes designated synthetic reactions) are conjugation to glucuronic acid, sulfate glutathione, etc. Originally, phase I metabolism followed by phase II metabolism was considered to be the rule. It is now realized that many compounds undergo only one of the phases. From a toxicological point of view there are only very few examples of drugs that are activated into toxic metabolites by phase II metabolism whereas phase I metabolism can frequently yield toxic metabolites. One such example is paracetamol (acetaminophen) to be discussed later in this paper.

In man the CYP (cytochrome P450) isozymes are the major catalysts of drug oxidation, and within the last decade considerable progress has been made in identifying the genetic basis for the huge intraindividual variation in drug metabolism. Although genetic polymorphisms are recognized as major determinants of individual variation in the activities of drug metabolizing enzymes it must be stressed that also with regard to non-polymorphous enzymes large interindividual variability with equally important implications can be found.

In the context of metabolizing chemicals a genetic polymorphism is the presence within a population of at least two groups with distinctly different ability of metabolizing xenobiotics. Presently, a polymorphism is defined by a frequency in the population of at least 1% of at least two phenotypes (Vogel and Motulsky 1982). In this review special focus is on the relevance of genetic polymorphisms in drug metabolism for the risk assessment of drugs.

Genetic polymorphisms in man:

CYP2D6:

CYP2D6 is found in human liver (Gonzalez et al. 1988) and in the brain (Tyndale et al. 1991). A polymorphism in oxidation of the antihypertensive drug debrisoquine was observed (Mahgoub et al. 1977) because of a particular slow elimination and pronounced pharmacodynamic effect in about 8% of the caucasian population. Many

drugs are now realized to be oxidized by CYP2D6 (Brøsen and Gram 1989).

The genetic defects in the CYP2D6 gene are well characterized (Kagimoto et al. 1990, Gaedig et al. 1991). Furthermore, the phenotype for debrisoquine hydroxylation can be predicted by DNA-testing (Graf et al. 1992).

Phenotyping: Sparteine, debrisoquine or dextromethorphan (Schmid et al. 1985) can be used as probes for phenotyping individuals. Poor metabolizers (PM) are defined from a metabolic ratio (parent compound/metabolite) in urine of >20 for sparteine (Eichelbaum et al. 1982) , > 12.6 for debrisoquine (Evans et al. 1980) and >0.3 for dextromethorphan (Schmid et al. 1985).

Genotyping: DNA obtained from leucocyte preparation after a conventional venesection can be used for PCR based determination of the CYP2D6 mutations D6-A, D6-B and D6-C (Heim and Meyer 1992).

Example: Codeine is an old analgesic used in many composite analgesic formulations. It is activated to morphine by CYP2D6 (Dayer et al. 1988) and its analgesic action is dependent on biotransformation. It is intriguing that while poor metabolizers have no analgesic effect of codeine fast metabolizers with quinidine induced poor metabolizer phenotype still have the analgesic effect (Weinshilboum and Sladek 1980, Sindrup et al. 1990). Presumably, local bioactivation of codeine in the brain plays a role (Wahlström et al. 1988) and this mechanism may not be inhibited by quinidine.

CYP1A 1/2:

CYP1A1 has been detected in human placenta, skin, lymphocytes and peripheral lung, whereas it is notably absent in human liver (Kadlubar and Guengerich 1992). This enzyme is among the best conserved among xenobiotic-metabolizing enzymes (Nebert 1989). The only available clinically example of a drug that is metabolized by CYP1A1 is coal tar used by dermatologists, the major focus on this enzyme is its role in activation of carcinogens and teratogens (Nebert 1989). In this respect it is noteworthy that the enzyme constitutively has a low expression and that many substances can induce its activity many fold, e.g. cigarette smoke, polyaromatic hydrocarbons etc. In relation to drug metabolism the most interesting feature is whether drugs have CYP1A1 inducing properties that might change the susceptibility of the treated individual to carcinogens or mutagens. In such a case long term treatment might increase the teratogenic or carcinogenic risk.

CYP1A2 has only been identified in the liver and there appears to be a 50 to 100-fold variability in its activity (Guengerich and Shimada 1991). CYP1A2 is important for the metabolism of important drugs, e.g. caffeine, phenacetin and paracetamol, and for a large number of xenobiotics, e.g. heterocyclic amines. A polymorphism has been suggested from a ratio of urinary caffeine metabolites (Butler et al. 1992). However, in studies using the conventional caffeine metabolic ratio on more than 300 persons such a polymorphism could not be detected (Vistisen et al. 1992). The conventional CYP1A2 index that correlates well with the caffeine clearance is between the two step demethylated metabolites of caffeine, i.e. 1-methylxanthine (1X), 1 methyluric acid (1U) and 5-acetyl-6-formylamino-3-methyl uracil (AFMU), and the one step demethy-

lated metabolite 1,7-dimethyluric acid (17U) (Campbell,ME et al. 1987). As stated below genotyping does not suggest any polymorphisms in CYP1A2. Presently it must be concluded that a polymorphism for CYP1A2 still needs to be verified.

Phenotyping: There are no available non-hazardous probes for determination of CYP-1A1 activity *in vivo, in vitro* ethoxyresorufin appears to be a specific probe (Jensen et al. 1993).

CYP1A2 activity can be estimated from dietary caffeine metabolite rations in urine as described above.

Genotyping: DNA from various tissues can, with or without the PCR amplification be used to determine restriction fragment length polymorphisms in CYP1A1 (Kawajiri et al. 1990, Tefre et al. 1991).

CYP1A2 protein analysis of human liver has demonstrated variations, but no liver has been found that lacks this enzyme suggesting that mutant alleles of CYP1A2 are not present in humans (Wrighton et al. 1986, McManus et al. 1988, Shimada et al. 1989).

Example: So far the only example of a drug with CYP1A1 inducing properties is coal tar for topical application whereas the environmentally dominating source of such xenobiotics are tobacco smoke. An association of lung cancer with CYP1A1 polymorphism (RFLP) has been found in a japanese population (Kawajiri et al. 1990) whereas this association could not be found in a Norwegian study (Tefre et al. 1991).

Regarding CYP1A2 a distinct polymorphism remains to be demonstrated, however, the 50-100 fold variability could be equally important for a variety of drug and xenobiotic related effects. A recent example presumably of clinical importance is the inhibitory effect of the antidepressant fluvoxamine as demonstrated by a low K_i for inhibition of CYP1A2 in human microsomes (Brøsen et al. 1993b).

CYP2E1:

In humans CYP2E1 is found in the liver and in peripheral blood lymphocytes. An approximate 10-fold variation in hepatic enzyme activity has been reported (Peter et al. 1990). CYP2E1 was originally called MEOS (microsomal ethanol oxidizing system) and it metabolizes ethanol and other structurally related compounds as for example some of the volatile anesthetics (halothane). The muscle relaxant chlorzoxazone (Peter et al. 1990) is metabolized almost exclusively by CYP2E1 and excreted after glucuronidation into urine, and the analgesic paracetamol (acetaminophen) is activated by CYP2E1 to its arylating metabolite. Presently, there are no systematic investigations available for comparison with the restriction fragment length polymorphisms identified for CYP2E1.

Phenotyping: The only suggested probe for CYP2E1 is chlorzoxazone (Peter et al. 1990). Preliminary investigations in our laboratory show that recovery of the 6-OH metabolite is almost complete in urine and that plasma clearance is about 300 ml/min. It would therefore be possible to use the clearance as a measure of CYP2E1 activity and to develop a simplified one sample method in analogy with the one developed for antipyrine (Poulsen and Loft 1988).

Genotyping: Recently, two reports have described restriction fragment length polymorphisms of CYP2E1. One is based on PstI or RsaI restriction and localized to the transcription regulation region of CYP2E1 (Hayashi et al. 1991). The other polymorphism is localized to entron 6 and revealed by the DraI restriction enzyme (Uematsu et al. 1991).

Example: Paracetamol is activated to an arylating metabolite by CYP2E1, CYP1A2 and CYP3A4 (Thummel et al. 1992, Poulsen et al. 1993) following an overdose that depletes hepatic glutathione. Preliminary investigations in our laboratory points at CYP2E1 as the qualitatively important activating route in mice (unpublished observations), in man, however, another enzyme could be the most important. It is still debated if e.g. alcoholism is a risk factor for development of hepatic injury from paracetamol overdose, since CYP2E1is thought to be induced. However, systematic investigations on this important clinical problem has yet to be done.

CYP2C.

This cytochrome (often called cytochrome $P450_{MF}$) is controlled by an autosomal recessive gene resulting in its absence in approximately 2-3% of the caucasian population as opposed to about 13% in orientals (Bertilsson et al. 1992). An increasing number of drugs are recognized to be metabolized by CYP2C, e.g. diazepam (Bertilsson et al. 1992), omeprazole (Rost et al. 1992) and proguanil (Ward et al. 1991, Funck-Brentano et al. 1992).

Phenotyping: The simplest approach to determine the phenotype appears to be the ratio between the (*R*)- and the (*S*)-enantiomers in urine collected for 8 hours after oral administration of the racemate (Wilkinson et al. 1989).

Genotyping: Presently there are no available methods.

Example: The antimalarial drug proguanil is metabolized by CYP2C to cylcoguanil (Ward et al. 1991), which is responsible for the antimalarial action. Poor metabolizers have only about 25% of the cycloguanil plasma level found in fast metabolizers (Brøsen et al. 1993a), whether this is reflected by a higher risk of therapeutic failure remains to be established.

N-acetyl transferase:

This enzyme is found in many tissues but is most abundant in the liver (Hein et al. 1987). It has been recognized for more than 30 years as genetically determined by two co-dominant alleles (Evans et al. 1960). The acetylation phenotypes, i.e. fast and slow acetylators, predict the rate of elimination of many drugs metabolized by this enzyme, e.g. isoniazid, procaineamide, sulfamethazine, nitrazepam, caffeine (Lou 1990). Both O- and N-acetylation of hydroxylamines are under the same genetic control and the two reactions are important for the metabolic activation and deactivation of environmental carcinogens, e.g. arylamines (Hein 1988).

The genetic defects are now well characterized. The dominant, autosomal allele for fast acetylators and the multiple alleles for slow acetylators have been located to chromosome 8 (Blum et al. 1990, Ohsako and Deguchi 1990).

Phenotype determination: Acetylator phenotype may be determined by probing with caffeine, isoniazid or sulfadimidine (Clark 1985). It should be noted, however, that some drugs are metabolized equally well by acetylation regardless of the phenotype, e.g. *p*-aminosalicylate (Weber and Hein 1985). By means of dietary caffeine intake acetylator phenotype can be determined from the urinary ratio between the acetylated metabolite 5-acetyl-6-formylamino-3-methyluracil (AFMU) and the alternative metabolite 1-methylxanthine(1X), or 1X plus 1-methyluric acid (1U) plus 1,7 demethyluric acid (17U). This index discriminates the two phenotypes in accordance with other indices (Grant et al. 1983, 1984). Whether this probing methodology can distinguish between homozygous and heterozygous fast acetylators is debated (Grant et al. 1983, 1984, Vistisen et al. 1992).

Genotype determination: Genomic DNA obtained from peripheral blood leukocytes can be used for a polymerase chain reaction based test for determination of the wild type (wt) and the mutated genes (M1, M2 and M3) (Graf et al. 1992). By this technique homozygous and heterozygous fast acetylators can be distinguished from slow acetylator genotypes.

Example: Weber et al. (Weber and Hein 1985) concluded that slow acetylators are more likely to develop peripheral neuropathy than fast acetylators from isoniazid, and patients suffering from isoniazid hepatitis have a higher frequency of hepatic disorders if they are fast acetylators. Further supporting this hypothesis is the higher prevalence of isoniazid-induced liver toxicity in orientals (fast acetylators constitute 80 - 90% of the population) than in caucasians (fast acetylators constitute 40 -50% of the population).

Nevertheless, the main importance regarding this polymorphism is probably related to environmental and occupational exposure to aromatic amines and related cancer risk (Hein 1988).

Sulfoxidation.

Sulfoxidation is suggested to be genetically polymorphic regulated. However, solid evidence on the protein and gene level is still not available. A number of sulfur containing drugs are eliminated by sulfoxidation. The formation of a metabolite from the mucolytic agent carbocisteine as assessed by a urinary metabolic ratio has appeared to be under genetic control (Mitchell et al. 1984). Although a low rate of metabolite excretion has been associated with various inflammatory and neurological diseases (Steventon et al. 1990, Gordon et al. 1992), it has only been related to a few reactions to sulfur containing drugs, i.e.D-penicillamine (Emery et al. 1984), and this could be due to an apparently worse clinical course in patients with this trait (Emery et al. 1992). In fact, the claim that the putative carbocisteine metabolite is a product of sulfoxidation and its importance have been disputed by others (Brockmoller et al. 1991, Meese et al. 1991, Gregory et al. 1992). No definite polymorphism with respect to sulfoxidation of drugs has yet been identified.

Methylation.

This reaction lacks the in depth investigations that are available for the CYPs. However, conjugation of methyl groups to thiols, oxygen and nitrogen appear to be under separate genetic control (Weinshilboum 1989, 1992). Thiopurine methyltransferase (TPMT), a cytoplasmatic enzyme expressed in all tissues, catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl compounds, including 6-mercaptopurine and azathioprine. The activity of TPMT may be assessed from red blood cells. From population and family studies the activity appears to be controlled by a single gene with two alleles and gene frequencies for high and low activity of 94% and 6%, respectively (Weinshilboum 1992). Thus, in 298 subjects 88.6%, 11.1% and 0.3% had high, intermediate, and no activity, respectively (Weinshilboum and Sladek 1980). Whereas patients with low TPMT activity may suffer toxicity to the thiopurine drugs (Lennard et al. 1987, 1989), those with particularly high activity may risk underdosing and relaps more frequently when treated for leukemia (Lennard et al. 1990).

Thiolmethyltransferase (TMT), a membrane bound enzyme expressed in most tissues, catalyzes the methylation of aliphatic sulphydryl drugs, such as captopril and D-penicillamine (Weinshilboum 1989, 1992). The activity as assessed from red blood cells appear to be under some genetic control. Although particular TMT activities have been associated with some neurological diseases (Waring et al. 1989), no relationship with any drug reaction has been reported.

Cytoplasmic cathecol O-methyltransferase (COMT) metabolizes some neurotransmitters and catechol drugs (Weinshilboum 1989, 1992). The distribution of COMT activity assessed from red blood cells in a population suggests regulation by a single locus with two alleles and almost equal gene frequencies. Whereas COMT activity correlates with the rate of metabolism of L-dopa and methyldopa (Reilly et al. 1980, Campbell,NRC et al. 1984), the clinical significance has yet to be demonstrated.

From a large family study the activity of N-methyltransferase expressed in red blood cells appears to be under genetic control (Scott et al. 1988). Although histamine is N-methylated by this enzyme, no drug has yet been identified as an important substrate.

Sulfation:

Sulfotransferases belong to a family of enzymes found mainly in the cytosol of hepatocytes but also in platelets. In contrast to the CYPs they are not investigated in the same details regarding the proteins and the genes.

Both thermolabile and thermostable phenol sulfotransferase as assessed from platelets appear to be under genetic controls in population and family studies (Weinshilboum 1990). The extent of paracetamol sulfation correlated with the platelet activity of both forms in one study (Reiter and Weinshilboum 1982) but only with the thermostable form in another study (Bonham-Carter et al. 1983). In subjects supplemented with sodium sulfate the extent of methyldopa sulfation correlated with only the thermolabile sulfotransferase activity of platelets (Campbell,NRC et al. 1985), although

this does not predict the activity of this form in other tissues (Weinshilboum 1990). The clinical significance of genetic variation in the sulfation capacity, which may also be dependent on (activated) sulfate availability has yet to be determined.

Miscellaneous reactions:
A variety of other reactions are not reviewed in details here because they either are consdered irrelevant for drug metabolism, because they represent particular problems that should be addressed separately in the risk assessment of drugs (e.g. the hepatic cytosolic alcohol dehydrogenase) or because only very sporadic information is available (e.g. some of the CYPs, the paroxonase/arylesterase polymorphisms) on the proteins and genes.

Phenotyping versus genotyping:

Several consideration on the choice between phenotyping and genotyping can be made although it should be realized that the two methods are supplementary rather that alternatives. Phenotyping has the advantage of continuous scale measurement and can therefore determine potential drug interactions. For example will quinidine administration turn fast CYP2D6 metabolizers phenotype to slow metabolizer phenotype (Sindrup et al. 1992), an effect that cannot be revealed by genotyping. Analogous arguments are valid for inducing effects. Furthermore, most polymorphisms show a considerable variability even in one of the genotypes. Very fast fast-metabolizers for example have a enzyme activity that cannot be distinguished from slow fast-metabolizers by genotyping, whereas phenotyping offers the possibility of revealing reactions related to such a trait. Furthermore, it can be argued that the huge variability that can be found in non-polymorphic enzyme activities can be of equal magnitude and importance for administration of drugs. The draw-back of probing is that it requires a trial that is planned for this purpose and that it requires administration of a test compound and sampling of relevant biological material.

The advantage of analysis on DNA for genotyping is that it only requires minute amounts of genomic DNA from e.g. lymphocytes because of the PCR technique and that it can be done retrospectively if such material is stored. Genotyping has a high potential for screening and the molecular techniques have a high capacity and a price that get cheeper and cheeper. It should be noted that only in case of CYP2D6 and N-acetyltranferase there are solid evidence that the genotyping predict the phenotype with sufficient accuracy. Interestingly, multiple genes are said to be found in fast fast-metabolizers of debrisoquine.

Which polymorphisms are relevant for drug development and registration?

The polymorphisms in CYP2D6, CYP2C and N-acetyltransferase are clearly demonstrated of importance for the metabolism of many drugs. For a safety evaluation it is mandatory to include investigations on whether a new drug is metabolized by these enzymes. If it can be established that the metabolism by either of these pathways only

constitute a minor pathway further investigations are not necessary unless in the case(s) where the metabolism leads to conversion of a prodrug to an active metabolite, confer the codeine and proguanil examples mentioned above. The safety evaluation should also include whether a new drug has inhibiting effects on either of these enzymes, e.g. by estimating metabolism of probe drugs in human microsome systems or transfected cellular systems. For example we have recently shown that the antidepressant fluvoxamine is a potent inhibitor of CYP1A2 (Brøsen et al. 1993b). A similar effect has led to withdrawal of the drug furafylline due to its inhibition of caffeine metabolism leading to caffeine intoxication from dietary intake. Transfected cell lines can be used for specific elucidation of analogous effects (Jensen et al. 1993).

Recommendations:

In the safety evaluation of new drugs it should be examined whether or not they are metabolized by the enzymes that exhibit genetic polymorphism, i.e. CYP2D6, CYP2C and N-acetyltransferase. If this is the case further safety studies are required particularly if the drug has a steep dose response curve and/or the pharmacodynamic effects include influence on vital functions, i.e. a narrow therapeutic index. Particular emphasis should be put on drug that are activated by polymorphous enzymes, in which case even a minor metabolic route may be of considerable importance.

Regardless of the drug metabolism route it should be documented that new drugs do not inhibit the enzymes of the major drug metabolism polymorphisms. These recommendations can be extended to existing drugs as well and to other important routes of drug elimination.

If a new drug depends on bioactivation by CYP2D6 or CYP2C about 4 - 10% of the population would have no therapeutic effect while they still may suffer from the side-effects. If a new drug is metabolized by a genetic polymorphous enzyme it should be determined if drug action depends on this and/or if the partial clearance is of a sufficient magnitude to result in interindividual variation in drug elimination .

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References:

Bertilsson L, Lou YQ, Du YL, Liu Y, Kuang TY, Liao XM, Wang KY, Reviriego J, Iselius L, Sjoqvist F (1992) Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. Clin Pharmacol Ther 51:388-397

Blum M, Grant DM, McBride W, Heim M, Meyer UA (1990) Human arylamine N-Acetyltransferase genes: isolation, chromosomal localization, and functional expression. DNA and Cell Biology 9:193-203

Bonham-Carter SM, Rein G, Glover V, Sandler M, Caldwell J (1983) Human platelet phenol

sulphotransferase M and P: substrate specificity and correlation with in vivo sulphoconjugation of paracetamol and salicylamide. Br J Clin Pharmacol 15:323-330

Brockmoller J, Staffeldt B, Roots I (1991) Evaluation of proposed sulphoxidation pathways of carbocisteine in man by HPLC quantification. Eur J Clin Pharmacol 40:387-392

Brøsen K, Gram LF (1989) Clinical significance of the sparteine/debrisoquine oxidation polymorphism. Eur J Clin Pharmacol 36:537-547

Brøsen K, Skjelbo E, Flachs H (1993a) Proguanil metabolism is determined by the mephenytoin oxidation polymorphism in Vietnamise living in Denmark. Br J Clin Pharmacol in press:

Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft L (1993b) Fluvoxamine is a potent inhibitor of cytochrome P4501A2. Biochem Pharmacol 45:1211-1214

Butler MA, Lang NP, Young JF, Caporaso N, Vineis P, Hayes RB, Teitel CH, Massengrill JP, Lawsen MF, Kadlubar FF (1992) Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics 2:116-127

Campbell ME, Spielberg SP, Kalow W (1987) A urinary metabolite ratio that reflects systemic caffeine clearance. Clin Pharmacol Ther 42:157-165

Campbell NRC, Dunette JH, Mwaluko G, Van Loon J, Weinshilboum RM (1984) Platelet phenol sulfotransferase and erythorcyte catechol O-methyltransferase activities: correlation with methyldopa metabolism in man. Clin Pharmacol Ther 35:55-63

Campbell NRC, Sundaram RS, Werness PJ, Van Loon J, Weinshilboum RM (1985) Sulfate and methyldopa metabolism: metabolite patterns and phenol sulfotransferase activity. Clin Pharmacol Ther 37:308-315

Clark DWJ (1985) Genetically determined variability in acetylation and oxidation therapeutic implications. Drugs 29:342-375

Dayer P, Desmeules J, Leemann T, Striberni R (1988) Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation. Biochem Biophys Res Commun 152:411-416

Eichelbaum M, Bertilsson L, Säve J, Zekorn C (1982) Polymorphic oxidation of sparteine and debrisoquine: Related pharmacogentic entities. Clin Pharmacol Ther 31:184-186

Emery P, Panayi SP, Huston G, Welsh KI, Mitchell SC, Smith RL, Waring RH (1984) D-penicillamine induced toxicity in rheumatoid arthritis: the role of sulphoxidation status and HLA-DR3. J Rheumatol 11:626-632

Emery P, Bradley H, Arthur V, Tunn E, Waring R (1992) Genetic factors influencing the outcome of early arthritis - the role of sulphoxidation status. Br J Rheumatol 31:449-451

Evans DAP, Manley KA, McKusick VA (1960) Genetic control of izoniazid metabolism in man. Br Med J 2:485-491

Evans DAP, Mahgoub A, Sloan TP, Idle JR, Smith RL (1980) A family and population study og genetic polymorphism of debrisoquine oxidation in a white British population. J Med Genet 17:102-105

Funck-Brentano C, Bosco O, Jacqz-Aigrain E, Keundjian A, Jaillon P (1992) Relation between chloroguanide bioactivation to cycloguanil and the genetically determined metabolism of mephenytoin in humans. Clin Pharmacol Ther 51:507-512

Gaedig KA, Blum M, Gaedig KR, Eichelbaum M, Meyer UA (1991) Deletion of the entire cytochrome P450 CYP2D6 geneas a cause of impaired drug metabolism in poor metabolzers of the debrisoquine/sparteine polymorphism. Am J Human Genet 48:943-950

Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP, Meyer UA (1988) Characterization of the common genetic defects in humans deficient in debrisoquine metabolism. Nature 331:442-446

Gordon C, Bradley H, Waring RH, Emery P (1992) Abnormal sulphur oxidation in systemic lupus erythematosus. Lancet 339:25-26

Graf T, Broly F, Hoffmann F, Probst M, Meyer UA, Howald H (1992) Prediction of the phenotype for acetylation and for debrisoquine hydroxylation by DNA-tests in healthy human volunteers. Eur J Clin Pharmacol 43:399-403

Grant DM, Tang BK, Kalow W (1983) Variability in caffeine metabolism. Clin Pharmacol Ther 33:591-602

--- (1984) A simple test for acetylator phenotype using caffeine. Brit J Clin Pharmacol 17:459-464

Gregory WL, James OF, Idle JR (1992) Carbocisteine polymorphism and disease. Lancet 339:616-617

Guengerich FP, Shimada T (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chemical Research in Toxicology 4:391-407

Hayashi S, Watanabe J, Kawajiri K (1991) Genetic polymorphisms in the 5'-flanking region change transscriptional regulation of the human cytochrome P450IIE1 gene. Biochem J 110:559-565

Heim MH, Meyer UA (1992) Evolution of a highly polymorphic human cytochrome P450 gene cluster: CYP2D6. Genomics 14:49-58

Hein DW (1988) Acetylator genotype and arylamine-induced carcinogenesis. Biochim Biophys Acta 948:37-66

Hein DW, Flammang TJ, Kirlin WG, Trinidad A, Ogolla F (1987) Acetylator genotype-dependent metabolic activation of carcinogenic N-hydroxylaamines by S-acetyl coenzyme A-dependent enzymes of inbred hamster tissue cytosols: relationship to arylamine N-acetyltransferase. Carcinogenesis 8:1767-1774

Jensen KG, Önfelt A, Poulsen HE, Doehmer J, Loft S (1993) Effects of benzo[a]pyrene and ±-trans-7,7-dihydroxy-7,8-dihydro-benzo[a]pyrene on mitosis in Chinese hamster V79 cells with stable expression of rat cytochrome P4501A1 or 1A2. Carcinogenesis in press:

Kadlubar FF, Guengerich PF (1992) Inducibility of human cytochromes P-450 primarely involved in the activation of chemical carcinogens. Chemosphere 25:201-204

Kagimoto M, Heim M, Kagimoto K, Zengin T, Meyer UA (1990) Multiple mutations of human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functinal significance of individual mutation by expression of chimeric genes. J Biol Chem 265:17209-17214

Kawajiri K, Nakachi K, Imai K, Shinoda N, Watanabe J (1990) Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P4501A1 gene. FEBS Lett 263:131-133

Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM (1987) Thipopurine pharmacogenetics in leukemia: correlation of erythrocyte methyltransferase activity and 6-thioguanine nucleotide concentrations. Clin Pharmacol Ther 41:18-25

Lennard L, Van Loon JA, Weinshilboum RM (1989) Pharmacogenetics of acute azathioprine toxocity: relationship to thiopurine methyltransferase genetic polymorphism. Clin Pharmacol Ther 46:149-154

Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM (1990) Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. Lancet 336:225-229

Lou YC (1990) Differences in drug metabolism polymorphism between orientals and caucasians. Drug Metab Rev 22:451-475

Mahgoub A, Idle JF, Dring LG, Lancaster R, Smith RL (1977) Polymorphic hydroxylation of

debrisoquine in man. Lancet 2:584-586

McManus ME, Stupans I, Ioannoni B, Burgess W, Robson RA, Birkett DJ (1988) Identification and quantitation in human liver of cytochromes P-450 analogous to rabbit cytochromes P-450 forms 4 and 6. Xenobiotica 18:207-216

Meese CO, Fischer C, Kupfer A, Wisser H, Eichelbaum M (1991) Identification of the "major" polymorphic carbocysteine metabolite as S-(carboxymethylthio)-L-cysteine. Biochem Pharmacol 42:R13-R16

Mitchell SC, Waring RH, Haley CS, Idle JF, Smith RL (1984) Genetic aspects of the polymodally distributed sulfoxidation of S-carboxymethyl-L-cysteine in man. Brit J Clin Pharmacol 18:507-521

Nebert DW (1989) The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. Crit Rev Toxicol 20:153-174

Ohsako S, Deguchi T (1990) Cloning and expression of cDNAsfor polymorphic and monomorphic arylamine N-acetyltransferases from human liver. J Biol Chem 265:4630-4634

Peter R, Böcker R, Beaune PH, Iwasaki M, Guengerich FP, Yang CS (1990) Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIE1. Chem Res Toxicol 3:566-573

Poulsen HE, Loft S (1988) Antipyrine as a model drug to study hepatic drug-metabolizing capacity. J Hepatol 6:374-382

Poulsen HE; Roberts DW, Hinson JA (1993) Covalent binding of acetaminophen estimated from protein adducts. In: Jeffery EH (ed) Human Drug Metabolism. From molecular biology to man. CRC Press, Boca Raton, Ann Arbor, London, Tokyo, pp. 43-52

Reilly DK, Rivera-Calimlim L, Van Dyke D (1980) Cathecol O-methyltransferase activity: a determinant of levodopa response. Clin Pharmacol Ther 28:278-286

Reiter C, Weinshilboum R (1982) Platelet sulfotransferase activity: correlation with sulfate conjugation of acetaminophen in man. Clin Pharmacol Ther 32:612-621

Rost KL, Brosicke H, Brockmoller J, Scheffler M, Helge H, Roots I (1992) Increase of cytochrome P450IA2 activity by omeprazole: evidence by the 13C-[N-3-methyl]-caffeine breath test in poor and extensive metabolizers of S-mephenytoin. Clin Pharmacol Ther 52:170-180

Schmid B, Bircher J, Preisig R, Kupfer A (1985) Polymorphic dextromethorphan metabolism Co-segregation of oxidative O-demethylation with debrisoquine hydroxylation. Clin Pharmacol Ther 38:618-624

Scott MC, Van Loon JA, Weinshilboum RM (1988) Pharmacogenetics of N-methylation: heritability of human erythrocyte N-methyltransferase activity. Clin Pharmacol Ther 43:256-262

Shimada T, Iwasaki M, Martin MV, Guengerich FP (1989) Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by umu gene response in Salmonella typhimurium TA 1535/pSK1002. Cancer Res 49:3218-3228

Sindrup SH, Brosen K, Bjerring P, Arendt-Nielsen L, Larsen U, Angelo HR, Gram LF (1990) Codeine increases pain thresholds to copper vapor laser stimuli in extensive but not poor metabolizers of sparteine. Clinical Pharmacology & Therapeutics 48:686-693

Sindrup SH, Arendt-Nielsen L, Brosen K, Bjerring P, Angelo HR, Eriksen B, Gram LF (1992) The effect of quinidine on the analgesic effect of codeine. European Journal of Clinical Pharmacology 42:587-591

Steventon GB, Heafield MTE, Sturman S, Waring RH, Williams AC (1990) Xenobiotic metabolism in Alzheimer's disease. Neurology 40:1095-1098

Tefre T, Ryberg D, Haugen A, Nebert DW, Skaug V, Brøgger A, Børresen A-L (1991) Human CYP1A1 (cytochrome P450) gene: lack of association between the Msp I restriction fragment length polymorphism and incidence of lung cancer in a Norwegeian population. Pharmacogenetics 1:20-25

Thummel KE, Lee CA, Kunze KL, Nelson;SD, Slattery JT (1992) Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochem Pharmacol 45:1563-1569

Tyndale RF, Sunahara R, Inaba T, Kalow W, Gonzalez FJ, Niznik HB (1991) Neuronal cytochrome P450IID1 (debrisoquine/sparteine-type): potent inhibition of activity by (-)-cocaine and nucleotide sequence identity to human hepatic P450 gene CYP2D6. Molecular Pharmacology 40:63-68

Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, Wakui A, Kanamaru R, Watanabe M (1991) Association between restriction fragment length polymorphism of the human cytochrome P450IIE1 gene and susceptibility to lung cancer. Jpn J Cancer Res 82:254-256

Vistisen K, Loft S, Poulsen HE (1992) Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. Carcinogenesis 13:1561-1568

Vogel F, Motulsky AG (1982) Human Genetics, problems and approaches. Springer-Verlag,Berlin, Heidelberg, New York

Wahlström A, Winblad B, Bixo M, Rane A (1988) Human brain metabolism of morphine and naloxone. Pain 35:121-127

Ward SA, Helsby NA, Skjelbo E, Brøsen K, Gram LF, Breckenridge AM (1991) The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism - a panel study. Br J Clin Pharm 31:689-692

Waring RH, Sturman SG, Smith MCG, Steventon GB, Heatfield MTE, Williams AC (1989) S-Methylation in motorneuron disease and Parkinson's disease. Lancet ii:356-357

Weber WW, Hein DW (1985) N-acetylation pharmacogenetics. Pharmacol Rev 37:25-77

Weinshilboum R (1989) Methyltransferase pharmacogenetics. Pharmac Ther 43:77-90

--- (1990) Sulfotransferase pharmacogenetics. Pharmacol Ther 45:93-107

Weinshilboum RM (1992) Methylation pharmacogenetics: thiopurine methyltransferase as a model system. Xenobiotica 22:1055-1071

Weinshilboum RM, Sladek S (1980) Mercaptopurine pharmacogenetics: monogene inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 32:651-662

Wilkinson GR, Guengerich FP, Branch RA (1989) Genetic polymorphism of S-mephenytoin hydroxylation. Pharmacol Therap 43:53-76

Wrighton SA, Campanile C, Thomas PE, Maines SL, Watkins PB, Parker G, Mendez-Picon G, Haniu M, Shively JE, Levin W (1986) Identification of a human liver cytochrome P-450 homologous to the major isosafrole-inducible cytochrome P-450 in the rat. Mol Pharmacol 29:405-410