Variability in Human Drug Response
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Pharmacogenetic basis of variation in drug dependence

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ABSTRACT

Pharmacogenetic variations in the patterns of metabolism among individuals can importantly modulate the risk of drug dependence. Cytochrome P450 drug metabolizing enzymes (CYPs), can "activate" drugs of abuse (e.g. codeine to morphine) or deactivate drugs (e.g. nicotine to cotinine). Some CYPs are polymorphic, that is, there are gene mutations which result in no active enzyme (null mutations). Individuals with two null mutations appear in the population as phenotypic "poor metabolizers".

Using *in vitro* studies, we have identified drugs of abuse that are substrates of the polymorphic enzymes CYP2D6, CYP2A6 and CYP2C19. In human experimental studies, we have shown that CYP phenotype and genotype affect abuse liability for CYP2D6 metabolized drugs of abuse. In addition, we inhibited CYP2D6 and decreased individuals' risk of dependence experimentally and in treating codeine dependence.

In epidemiologic studies CYP2D6 and CYP2A6 null mutations protect individuals from becoming codeine and tobacco dependent, respectively. With respect to CYP2A6, heterozygote individuals, if they become smokers, smoke about 25% fewer cigarettes because of their slower nicotine metabolism. Since normally occurring mutations in CYP alleles decrease the risk of dependence, pharmacologic modification of CYP activity has the potential to prevent and treat drug dependence.

Key words: CYP2A6, CYP2D6, CYP2C19, behaviour, tobacco.

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DRUG DEPENDENCE AS A MODEL OF BEHAVIOUR

Drug dependence is the consequence of the interaction of the drug, the individual and the environment. With respect to the drug, it must serve as a reinforcer by increasing the probability that once taken the drug will be taken again. Drugs that are reinforcers can have this action by having positive rewarding effects (e.g. subjective euphoria) or by removing punishing or unpleasant experiences (e.g. alleviating depression during the "crash" from cocaine). Drugs which have aversive properties are less likely to be abused unless tolerance to the unpleasant effects occurs. Other features of the drug, which are important, include the rate of absorption and entry into the brain, lipid solubility and pattern of metabolism. For some drugs of abuse the parent drug is principally responsible for the drugs reinforcing properties (e.g. cocaine, nicotine, and amphetamines) while for others the metabolites are most important (e.g. codeine conversion to morphine).

Even though drug dependence is complex, its study is quite straightforward since precise and well-validated measures of the behaviour exist. Extensive methodology exist for quantitating the frequency of drug taking (an excellent measure of the strength of the reinforcer), the objective and subjective consequences of drug use, the physiologic effects of drug and the socio-economic effects of drug abuse. For this reason drug dependence provides an unusually precise model with which to determine the consequences of variations in drug disposition on behaviour.

THE CYTOCHROME P450 POLYMORPHISMS

Cytochromes (CYP) P450 (P450s) are enzymes involved in the oxidative metabolism of a wide array of endogenous and exogenous molecules, including steroids, plant metabolites, prostaglandins, biogenic amines, drugs and chemical carcinogens. This broad spectrum of reactions is due to multiple P450 isozymes, with differing but overlapping substrate specificities. The mammalian P450 super-family consists of at least 12 families and over 400 individual genes. Genetic variants of P450 have been discovered because of atypical clinical responses in individuals who were shown subsequently to have a reduced ability to metabolize a drug. These phenotypically different individuals (also called poor metabolizers [PMs]) have been the basis of identifying the genetic basis of the variants and denoting pharmacogenetic polymorphisms. Those with enzyme activity are termed extensive metabolizers (EMs). The phenotype of individuals is determined along with the activity of CYPs using a sub-clinical dose of a probe drug such as dextromethorphan (CYP2D6), omeprazole (CYP2C19) or coumarin (CYP2A6). The genotype of the individual is determined with allele specific polymerase chain reaction amplification assays developed for the detection of specific mutations in the CYP gene.

In addition, to the most commonly occurring or normal ("wild type") allele, for most CYPs, allele variants have been identified that result in the gene producing an inactive enzyme (null mutation) or an enzyme with lower or even higher activity. As a result in any population combinations of homozygote wild type and null mutations exist along with the heterozygotes for all allele variants. With respect to nomenclature the wild type is denoted as the *1 allele and subsequent allele variants are denoted as *2, *3, *4, etc. based on their order of discovery.

For CYPs with null mutations the frequency-distribution of the logarithm of the ratio of parent drug to metabolite may be bimodal with varying percentages of the population comprising the upper mode with low activity (PMs). The frequency of the PM phenotype and allele frequencies shows considerable inter-ethnic variation [1].

IMPACT OF PHARMACOGENETIC POLYMORPHISMS ON DEPENDENCE RISK

Because these cytochrome are involved in the metabolism of drugs of abuse it is likely that differences in patterns of metabolism among individuals and across ethnic group will importantly affect the risk of drug abuse and dependence (e.g. CYP2D6 codeine, hydrocodone, p-methoxyamphetamine, amphetamine) or inhibitors (e.g. (-)-cocaine, pentazocine; CYP2A6 nicotine; CYP2C19 diazepam and other benzodiazepines). The consequences of absent or inhibited CYP activity for any particular drug will depend on the relative activity of the parent drug and its various metabolites. In some cases, the pharmacology of the metabolite is qualitatively similar to the parent; in other cases, the pharmacology is different (e.g. dextromethorphan to dextrorphan); more usually, it is not properly understood. In addition, some CYPs occur within the CNS. Their role in the brain is unknown, but potential formation of active drug metabolites at their site of action makes the presence of them potentially of great functional significance. A computer simulation study of inter-regional differences in CNS drug metabolism has provided a model which could account, in part, for large inter-subject variability in the pharmacodynamic effects of psychoactive drugs [2].

CYP deficient individuals should have a much decreased probability of abusing a drug converted to an active metabolite capable of maintaining drug-taking behaviour (e.g. codeine, oxycodone, hydrocodone, dextromethorphan). In EMs, the probability of dependence is increased, and proportionate to their genotype (homozygous versus heterozygous) and absolute CYP activity. Conversely, PMs should experience greater risk of abuse or of toxicity to a drug which is inactivated by a CYP and EMs a lesser risk. The clinical consequence of this could be either an increased risk or decreased risk of dependence depending on the relative punishing as versus reinforcing drug properties.

CYTOCHROME P450 2D6

Several drugs of abuse, codeine [3], hydrocodone, oxycodone [4], dextromethorphan [5], and p-methoxyamphetamine [6] are known to be metabolized by this enzyme.

i) **Amphetamines**

Deficiency in the p-hydroxylation of amphetamine was one of the observations that led to the discovery of the CYP2D6 polymorphism [7,8]. A single oral administration of the radiolabelled enantiomers of amphetamine to three volunteers with subsequent analysis of urine indicated that about 5% of (+)-amphetamine was converted to p-hydroxyamphetamine in two subjects but to a much less extent in a subject who was later found to have CYP2D6 deficiency [7,8].

The central nervous system stimulant p-methoxyamphetamine (PMA) is extremely O-demethylated by CYP2D6 to form 4-hydroxyamphetamine [6,9]. In the early 1970's, PMA

was associated with a number of idiosyncratic deaths in Canada [10]. A cardinal feature of these deaths was increased blood pressure, arrhythmias and hyperthermia. The lack of relationship to dose suggests that the individuals who died may have been CYPD6 (PMs). These clinical data and *in vitro* studies [11] suggest that CYP2D6 activity and individual genotype may contribute to amphetamine dependence risk and toxicity.

ii) **Dextromethorphan**

Dextromethorphan has the opposite steric configuration to codeine and morphine, is devoid of analgesic effects but is as potent an antitussive as codeine. It is the most commonly used antitussive world-wide. Systematic studies concerning its abuse liability are few. In the earliest report [12] no abuse liability was found with oral doses up to 100 mg. However, there are many case reports of dextromethorphan abuse and endemic use principally among young people [13]. Monkeys and rats can be trained to self administer dextromethorphan (with difficulty) but will do so more easily for its active metabolite dextrorphan. They will also recognize dextrorphan in particular as a discriminative stimuli and will generalize from and to phencyclidine [14] suggesting dextromethorphan abuse potential is due to its metabolite dextrorphan. Dextromethorphan is metabolized to its active metabolite dextrorphan by CYP2D6. Variations in the activity of the enzyme results in different kinetics and pharmacologic effects in EM and PM individuals [15,16]. EM individuals produce almost 40-fold more dextrorphan than PMs. In 4 EM and 2 PM normal volunteers given 3.0 mg/kg of dextromethorphan p.o. the effects of dextromethorphan are very different [16]. EMs show more euphoria and high effects and less sedation and dysphoria. As a result PM, individuals are probably protected from becoming dextromethorphan abusers by having greater punishing effects of the drug (sedation and dysphoria) and fewer pleasant effects (high and euphoria).

iii) **Codeine**

From a public health perspective, the misuse and abuse of opiate containing prescription medications is a poorly understood and largely unaddressed issue. Prescription analgesics including opioids (e.g. codeine, oxycodone, and hydrocodone) alone and in combination (e.g. 222®, Tylenol #3®) are extensively used worldwide and are always within the major classes of drugs prescribed in all countries.

Codeine is converted to morphine by CYP2D6. Morphine is at least 10-fold more potent than codeine as an analgesic and is responsible for the clinical efficacy of codeine and probably the side effects. In human experimental studies we have shown that CYP activity is a major determinant of codeine abuse liability [17]. In an epidemiologic study, the PM phenotype and genotype were absent among codeine dependent individuals compared to never dependent and dependent on other drugs of abuse comparison groups [18]. In addition, very high CYP2D6 activity was also not found among codeine dependent individuals suggesting that very extensive conversion to morphine (and its attendant side effects) might also protect against dependence.

CYTOCHROME P450 2A6

Recently a genetic polymorphism for CYP2A6 has been established PMs (lacking CYP2A6 activity) excrete less than 0.1% of the coumarin probe drug as 7-hydroxycoumarin [19,20].

Due to the recent identification of this polymorphism, the numbers of people who have been phenotyped/genotyped is small, however it appears that the allele frequency varies among ethnic groups.

i) **Nicotine Metabolism and Smoking Behaviour**

Nicotine is the primary compound present in tobacco that is responsible for establishing and maintaining tobacco dependence; dependent smokers adjust their smoking behaviour to maintain peripheral and central nicotine levels [21]. Evidence includes increased smoking behaviour if nicotine content in cigarettes is decreased, increased smoking if nicotine excretion is increased by urine acidification, decreased smoking with concurrent intravenous or patch IC [22]. Approximately 50% of initiation of smoking dependence is genetically influenced, while persistence of smoking and amount smoked have approximately a 70% genetic contribution. In humans, approximately 70% of nicotine is metabolized by inactivation to cotinine. We, and others, recently identified the genetically polymorphic CYP2A6 enzyme as the enzyme responsible for the majority of the metabolic conversion of nicotine to cotinine [23,24]. There is considerable interindividual variability in hepatic CYP2A6 function due predominantly to genetic variation in the *CYP2A6* gene locus. Three *CYP2A6* alleles have been identified: wild-type (*CYP2A6**1), and two defective null alleles (*CYP2A6**2 and *CYP2A6**3) [20]. Individuals with the *CYP2A6*2/ CYP2A6*3* and *CYP2A6*3/ CYP2A6*3* genotype have no CYP2A6-mediated metabolism.

Individuals with impaired nicotine metabolism (carriers of a defective *CYP2A6* allele[s]) would be protected from becoming tobacco-dependent. When learning to smoke individuals often find the nicotine unpleasant (e.g. causing dizziness or nausea). In individuals where nicotine metabolism was decreased, due to defects in the *CYP2A6* gene, the aversive effects might last longer or reach higher levels. Tobacco dependent (DSM-IV criteria) only, alcohol and tobacco dependent (DSM-IV criteria), and never-tobacco dependent groups were genotyped for *CYP2A6*. Twenty percent of the non-smoking population were carriers of defective *CYP2A6* alleles. In contrast to the non-smokers, in the dependent-smokers with or without alcohol dependence, only 12% of the individuals had *CYP2A6* defective alleles (20.1% [n = 213] versus 11.7% [n = 317], $p < 0.01$, χ-square; Odds Ratio = 1.9, 95% Confidence Intervals 1.2 - 3.2). This data provides evidence that impaired nicotine metabolism due to defective *CYP2A6* alleles is protective against becoming tobacco-dependent [25].

Within the tobacco-dependent group, those who had one defective and one active *CYP2A6* gene copy smoked significantly fewer cigarettes per day and per week than smokers without impaired nicotine metabolism ([carriers of two *CYP2A6* active alleles] 129 versus 159 cigarettes/week, t-test $p < 0.02$). These data show that heterozygosity in a single gene, the *CYP2A6* gene, significantly decreases both initiation of dependence and the drug-taking behaviour.

CYTOCHROME P450 2C19

The metabolism of mephenytoin and omeprazole are biomodal in the population, the deficiency is inherited at a single gene locus of CYP2C19. This CYP metabolizes many clinically used drugs including benzodiazepines. Ethnic differences in the PM frequency occur.

Three deficient variants from the active form of (*2, *3 and *4) have been identified; neither gene variant produces a functional CYP2C19 protein.

i) **Diazepam and Flunitrazepam Abuse**

Diazepam, is an established and important drug of abuse among opiate addicts, poly-drug abusers (including those with alcoholism), and young abusers worldwide especially in Asia [26]. However, flunitrazepam is the benzodiazepine with the highest abuse liability (followed by diazepam and is reported worldwide as the benzodiazepine most widely abused by opioid abusers [27]. Flunitrazepam is not marketed in the U.S. or Canada but is a growing and endemically abused drug in parts of the U.S. Two abuse liability experimental study of snorted flunitrazepam have shown increases in liking with increased doses of flunitrazepam. Subjective ratings and liking were highly correlated with flunitrazepam plasma levels [28]. Heroin users surveyed [27] reported using flunitrazepam only orally before 1990, but by 1994 36% of users reported injecting it i.v., usually in combination with heroin, allegedly to augment and prolong the subjective effects of heroin. Similarly, the fact that flunitrazepam tablets can be ground up and inhaled raises concern about toxicity and abuse. Heroin users or methadone patients have ranked flunitrazepam first in preference among benzodiazepines.

The mean clearance of diazepam and desmethyldiazepam is slower (about 40%) in the CYP2C19 PMs but there was considerable variability among individuals [29]. Inter-racial differences are likely due to concurrent differences in CYP2C19 and CYP3A. The higher frequency of 2C19 mutated gene in Asians and overrepresentation of heterozygous among Asian extensive metabolizers is one of the possible explanations for the observed differences both in clearance and subjective effects of diazepam. Omeprazole inhibits diazepam CYP2C19-mediated clearance and slows its elimination in Caucasian CYP2C19 EM [30]. Omeprazole (40 mg/ day for 21 days) decreased diazepam clearance by 28 ± 14.4 % and decreased desmethyldiazepam AUC by 42.4 ± 17% in Caucasians, which was significantly more than in Chinese (20% and 25% changes) [31].

The few studies of flunitrazepam metabolism have shown metabolism to desmethyl-flunitrazepam, 7-amino-flunitrazepam, 3-hydroxy-flunitrazepam and other aminobenzophenones. The compound mainly responsible for the hypnotic effect is thought to be the parent compound [32]; however, the N-desmethyl-metabolite also has affinity for the $GABA_A$ receptor complex [33]. Two studies have identified catalytic outliers who produced little or no N-desmethyl, consistent with CYP2C19 deficiency; one was an American Indian [32,34].

We now have preliminary evidence that genotype profoundly influences the objective and subjective effects of flunitrazepam in Chinese healthy volunteers. Two Chinese PMs and one EM subjects were administered flunitrazepam (1 mg, orally) after 8 h fasting period. Objective and subjective effects were then measured before and 0.5, 1, 2, 3, 4, 6 and 9 hours after flunitrazepam administration. Results showed that the PMs have significantly decreased psychomotor performance than the EMs. Also, sedation, elation and "spacey" and other subjective effects associated with benzodiazepine toxicity and abuse (e.g. drug liking, good effects, etc.) were significantly higher in PM compared to EM [35].

CONCLUSIONS

These studies establish that pharmacogenetic variations in drug metabolism have important behavioural consequences altering the risk of drug abuse and dependence. In addition, since these enzymes can be inhibited there are important implications with respect to the development of new and needed prevention and treatment approaches to this major public health problems.

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Discussion: Pharmacokinetic basis of variation in drug dependence

H.K. Kroemer:
Do you know whether there is a regulation of CYP2A6 by nicotine?

E.M. Sellers:
Although CYP2A6 is in a general a regulated enzyme, it does not appear to be regulated by nicotine in any important way.

H.K. Kroemer:
Is there any evidence about the extrahepatic expression of CYP2A6?

E.M. Sellers:
CYP2A6 is found in the lung and also in some other tissues like in the brain, although in quite small amounts. But where it is found it is very close to the mapping of the nicotinic receptors. It is different to the other cytochromes, whose distribution seems to be unrelated to anything we can figure out. At least, in this case, we can have some hypothesis about the relationship in the brain.

G.T. Tucker:
You probably won't answer this question, but I wonder which compound you have patented as an inhibitor of CYP2A6?

E.M. Sellers:
You are quite right, I am not able to answer that question.

A. Breckenrigde:
There are not a lot of acceptable compounds which might fulfil these criteria. Is that right?

E.M. Sellers:
I said there were not many substrates. There might be potent inhibitors, not necessary chemical inhibitors, which could alter the CYP2A6 expression by different mechanisms.

U. Klotz:
If your hypothesis on nicotine is right, then addiction to smoking should be much less in the Asian population. Did you look at some interethnic differences, or are there any epidemiological data?

E.M. Sellers:
We have not looked at this directly. An important issue related to this question is that counting cigarettes is not really the best way to quantitate smoking behaviour. It is necessary to use more precise measures of smoking behaviour and exposure to smoke. The same would be true with, for example, the exposure to carcinogenic compounds and their activation. Related to these issues, the Japanese population are an interesting because they, and particularly males, smoke a lot. Up to 60% of males are regular smokers. However, Japanese

have among the lowest incidence of lung cancer. This is an interesting kind of dichotomy, so there may be other factors such as the environmental contribution.

U. Klotz:
In the very small opioid dependent group of 83 patients, you did not see any poor metaboliser. According to a recent publication (Chen *et al.*, Br J Clin Pharmacol 1997) in a group of healthy volunteers participating in clinical studies, the poor metaboliser frequency was 1.6%. So with n = 83 you might be just within the confidence interval of spontaneous variation.

E.M. Sellers:
That is a possibility. These results need to be replicated by larger studies. Another factor we found quite important here was that our control populations have a lower frequency of the PM phenotype, from 3.5 to 4%. In different countries you see this. It requires very careful assessment of drug-dependence criteria and other drug abuse.

I.P. Hall:
On the nicotine story, can you just go over the difference between heterozygotes and homozygotes? What I need reassuring is that there is good functional evidence that the heterozygote genotype has a major functional effect.

E.M. Sellers:
In *in vitro* studies, when a bank of 31 genotyped livers were studied, the relationship of the CYP2A6 content to nicotine metabolism was highly correlated (r=0.96), therefore very little else appears to be contributing in the *in vitro* model system.

I.P. Hall:
If you take heterozygote smokers, matched for smoking exposure, they have got higher nicotine levels. Is that correct?

E.M. Sellers:
Yes, they do.