Minireview

GENETIC POLYMORPHISMS IN THE CYTOCHROME P450 2A6 (CYP2A6) GENE: IMPLICATIONS FOR INTERINDIVIDUAL DIFFERENCES IN NICOTINE METABOLISM

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ABSTRACT:

During the last couple of years, cytochrome P450 2A6 (CYP2A6; coumarin 7-hydroxylase) has received a lot of attention because it has been shown that it is the principle human nicotine C-oxidase. This enzyme also activates a number of structurally unrelated precarcinogens including many nitrosamines and aflatoxin B1, and metabolizes certain clinically used drugs. There is a pronounced interindividual and interethnic variability in CYP2A6 levels and activity, and much of this can be attributed to polymorphisms in the CYP2A6 gene, where a few inactivating mutations as well as gene deletions have been described. The frequency of the inactive alleles is low in European populations and very few poor metabolizers for the probe drug coumarin have been described in these populations. In contrast, a relatively high allele frequency (15–20%) of the CYP2A6 gene deletion has been found in Asians, resulting in a generally reduced activity in these populations. Because of the importance of CYP2A6 in nicotine metabolism, it has been suggested that the CYP2A6 genotype influences the individual differences in smoking behavior as well as lung cancer susceptibility. Several case-control studies have been conducted in this area, but these have yielded conflicting results. The recent progress in the field of CYP2A6 genetics and the development of more specific genotyping methods will facilitate molecular epidemiological studies aimed at clarifying these important issues.

There is pronounced interindividual and interethnic variability in the levels and activity of many drug metabolizing enzymes. This could cause individual differences in sensitivities to the effects and toxicity of many clinically used drugs and environmental compounds such as nicotine and precarcinogens. In many cases, the basis for this variability are genetic polymorphisms in the genes encoding these enzymes (Ingelman-Sundberg et al., 1999). One such enzyme is cytochrome P450 2A6 (CYP2A6), first identified as the human coumarin 7-hydroxylase (Miles et al., 1990; Yamano et al., 1990; Yun et al., 1991). This enzyme is predominantly expressed in the liver (Koskela et al., 1999) and is responsible for the clearance of many drugs and environmental chemicals. During the past couple of years our knowledge concerning this enzyme has substantially increased. Therefore the aim of the present review is to summarize the current knowledge about substrates, interindividual variability, and genetic polymorphism of this enzyme. Furthermore, the potential involvement of CYP2A6 in determining an individual’s smoking behavior and risk of lung cancer will be discussed.

CYP2A6 Substrate Specificity

A very specific reaction catalyzed by CYP2A6 is 7-hydroxylation of the naturally occurring plant compound coumarin, and a coumarin phenotyping test has therefore been developed to estimate an individual’s CYP2A6 in vivo activity (Rautio et al., 1992). CYP2A6 is also one of the major enzymes involved in human nicotine metabolism (see below) and the major enzyme responsible for the metabolism of the platelet-activating factor receptor antagonist SM-12502 [(+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidine-4-one hydrochloride] and the neuroprotective drug chlorothiazide. It also contributes to the metabolism of some other pharmaceuticals including methoxyflurane, halothane, valproic acid, and disulfiram, and can activate a number of precarcinogens e.g., aflatoxin B1, 1,3-butadiene, 2,6-dichlorobenzonitrile and the nitrosamines NNK [4-(methylamino)butylamine], NNA [4-(methylnitrosoamoio)-1-(3-pyridyl)-1-butanol], NDEA [N-nitrosodiethylamine], and NNN [N'-nitrosornicotine] (see Oscarson et al., 1998; Pelkonen et al., 2000 and references therein).

Interindividual Variability in CYP2A6 Activity

Early studies using human liver microsomes revealed a pronounced interindividual variability in CYP2A6 levels and activity, with some livers completely lacking the enzyme (Miles et al., 1990; Yun et al.,...
CYP2A13 cDNA was only recently cloned and expressed (Su et al., inactive (Yamano et al., 1990; Ding et al., 1995). A full-length enzyme has been shown to not incorporate heme and is therefore inactive because it does not incorporate heme (Yamano et al., 1990). It has also been suggested that decreased CYP2A6 activity in combination with decreased N-glucuronidation may contribute to this. As discussed below, a lot of the CYP2A6 variability can be attributed to polymorphisms in the CYP2A6 gene, but CYP2A6 activity is also modified by certain drugs and environmental factors. Thus, CYP2A6-dependent coumarin 7-hydroxylation is induced in vivo by several antiepileptic agents (Sotaniemi et al., 1995) and decreased CYP2A6 activity has been demonstrated in subjects with severe alcohol-induced liver cirrhosis (Sotaniemi et al., 1995) and viral hepatitis A (Pasanen et al., 1997).

Genetic Polymorphism of the CYP2A6 Gene

The CYP2A6 gene spans a region of approximately 6 kilobase pairs, contains 9 exons and has been mapped to the long arm of chromosome 19 (between 19q12 and 19q13.2) (Miles et al., 1989). It is located within a 350-kilobase pair gene cluster together with the CYP2A7 and CYP2A13 genes, two CYP2A7 pseudogenes, as well as genes in the CYP2B and CYP2F subfamilies (Hoffman et al., 1995). The CYP2A7 enzyme has been shown to not incorporate heme and is therefore inactive (Yamano et al., 1990; Ding et al., 1995). A full-length CYP2A13 cDNA was only recently cloned and expressed (Su et al., 2000), which revealed an enzyme active in NNK, coumarin, nicotine, and cotinine metabolism. CYP2A13 seems, however, to be expressed mainly in nasal mucosa, which limits its importance for total body clearance of CYP2A substrates.

Some of the interindividual variability in expression can be attributed to genetic polymorphisms in the CYP2A6 gene, and two variant alleles (CYP2A6*2 and CYP2A6*3) were described several years ago (Yamano et al., 1990; Fernandez-Salgueiro et al., 1995). The CYP2A6*2 allele encodes an enzyme with a L160H substitution, which is inactive because it does not incorporate heme (Yamano et al., 1990). It has also been suggested that the L160H substitution results in an enzyme with altered regiospecificity that catalyzes coumarin 3-hydroxylation instead of the usual 7-hydroxylation (Hadidi et al., 1997). A more likely explanation for this in vivo finding could, however, be a shunting of coumarin to the 3-hydroxylation pathway catalyzed by CYP1A1, CYP1A2, and CYP2E1, and CYP3A4 (Zhu et al., 1999) in the absence of CYP2A6-dependent 7-hydroxylation. The CYP2A6*3 allele was initially reported as a hybrid allele created through multiple gene conversions with the inactive CYP2A7 gene (Fernandez-Salgueiro et al., 1995) resulting in an allele where exons 3, 6, and 8 are of CYP2A7 origin. This allele is also considered to be inactive, although this has never been demonstrated. In a PM for coumarin 7-hydroxylation we detected an additional inactive allele, CYP2A6*5, where a highly conserved Gly-479 has been replaced with


3 In this review, I have used the nomenclature system for CYP2A6 alleles recommended by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (see http://www.imm.ki.se/CYPalleles).

valine (Oscarson et al., 1999a). When expressed in yeast, this substitution resulted in a very unstable enzyme devoid of catalytic activity, which is consistent with the PM phenotype.

Using the original genotyping method described to detect the CYP2A6*2 and CYP2A6*3 alleles, we noticed a poor correlation between the CYP2A6 genotype and phenotype determined in vivo using the probe drug coumarin. In particular, we found an individual who displayed coumarin 7-hydroxylation activity in vivo, although the subject was initially determined to be homozygous for the inactive CYP2A6*2 allele. We therefore developed a more specific method for the detection of these alleles, which gave a much better correlation with the phenotype (Oscarson et al., 1998). Using this method, we detected a CYP2A6*2 allele frequency of 1 to 3% in European populations, in contrast to the 15 to 17% previously reported (Fernandez-Salgueiro et al., 1995). Furthermore, we failed to detect any true CYP2A6*3 alleles (Oscarson et al., 1999a); a finding that has been supported by several other groups (Chen et al., 1999; Kitagawa et al., 1999; Sabol and Hamer, 1999). A CYP2A7 gene conversion in the 3' -flanking region of the CYP2A6 gene (CYP2A6*1B) that abolishes the binding site for one of the primers used in the earlier method is the likely explanation for most of these misclassifications (Oscarson et al., 1999a).

In Asian populations, earlier reports suggested that the frequency of the CYP2A6 PM phenotype was much higher than in Caucasians and an in vitro study using microsomes from Japanese subjects demonstrated that eight of 30 livers had very low or no CYP2A6 immuno-reactivity and activity (Shimada et al., 1996). Studies by Nunoya and coworkers (1998) suggested that a partial or whole deletion of the CYP2A6 gene could contribute to the PM phenotype in Japanese individuals. We studied this phenomenon using Southern blotting, PCR, and DNA sequencing, and described the structure of a CYP2A locus where the whole CYP2A6 gene has been deleted, resulting in abolished CYP2A6-dependent metabolism (Oscarson et al., 1999b). The origin of this locus appears to be an unequal crossover event between the 3' -flanking regions of the highly similar CYP2A6 and CYP2A7 genes (Nunoya et al., 1999; Oscarson et al., 1999b), resulting in a deletion allele (CYP2A6*4A) and an allele with two CYP2A6 genes in tandem (Rao et al., 2000) as the two reciprocal outcomes. In a Spanish individual, we also identified a slightly different type of CYP2A6 deletion allele (CYP2A6*4D), where the crossover region is instead located in intron 8 or exon 9 (Oscarson et al., 1999a). A PCR-based method for the detection of the CYP2A6*4 alleles was developed, and the allele frequency was found to be 15% in Chinese subjects, but only 1.0% in Finns and 0.5% in Spaniards (Oscarson et al., 1999b). It can be concluded that the CYP2A6*4 allele is one of the major defective alleles contributing to the PM phenotype in Asian populations.

A schematic overview of the different CYP2A6 alleles currently described is shown in Fig. 1, and Table 1 summarizes the allele frequencies in European and Asian populations. Taken together, it can be observed that there are multiple alleles present in the population, which are the result of unequal crossover events and/or gene conversions between the CYP2A6 and CYP2A7 genes, and care must therefore be taken when designing genotyping methods to avoid similar genotype misinterpretations such as those that occurred for the CYP2A6*3 allele. The high-throughput methods that are now becoming available for rapid screening of novel polymorphisms will likely reveal a number of additional polymorphisms in the CYP2A6 gene that may modulate CYP2A6 levels and activity.
Implications of the CYP2A6 Polymorphism for Nicotine Metabolism and Smoking Behavior

In humans, 70 to 80% of the nicotine dose is C-oxidized by cytochrome P450 to a nicotine Δ1(5')-iminium ion, which is further metabolized to cotinine by a cytosolic aldehyde oxidase. Nicotine is, however, also N-oxidized by flavine monooxygenases to nicotine N9-oxide. Cotinine is subsequently converted to a number of hydroxylated products including trans-3β-hydroxycotinine, 5β-hydroxycotinine, and norcotinine (Fig. 2). In addition, nicotine, cotinine, and trans-3β-hydroxycotinine are glucuronidated (see Benowitz and Jacob, 1997 for a review on nicotine metabolism). In vitro studies using human liver microsomes and recombinant P450s have shown that CYP2A6 is the most important P450 responsible for the C-oxidation of nicotine (Cashman et al., 1992; Nakajima et al., 1996b; Messina et al., 1997), and the nicotine clearance was decreased in liver microsomes from individuals heterozygous for the CYP2A6*2 allele (Inoue et al., 2000). Other P450s such as CYP2B6 can, however, also catalyze the C-oxidation of nicotine but with a lower affinity (Yamazaki et al., 1999). This is relevant especially in individuals who lack active CYP2A6 enzyme. CYP2A6 is also involved in the subsequent oxidation of cotinine to several different products (Nakajima et al., 1996a; Murphy et al., 1999), although the relative importance of different enzymes in catalyzing these reactions has not yet been fully elucidated. An in vivo study further stressed the importance of CYP2A6 in nicotine metabolism, as individuals homozygous for a CYP2A6 gene deletion displayed only 15% of urinary cotinine levels compared with individuals carrying at least one active CYP2A6 gene after smoking the same number of cigarettes (Kitagawa et al., 1999). Earlier reports suggesting a major contribution of CYP2D6 (debrisoquine hydroxylase) to the in vivo metabolism of nicotine have, however, not been reproduced (Benowitz et al., 1996), thereby questioning the role of this enzyme in nicotine metabolism.

Because of the substantial involvement of CYP2A6 in nicotine elimination, it has been proposed that the CYP2A6 polymorphism is a major determinant of an individual’s smoking behavior. It has also been suggested that CYP2A6 inhibitors can be used as a new approach to treat tobacco dependence (Sellers et al., 2000). Pianezza and coworkers (1998) showed that a lower number of individuals carrying the CYP2A6*2 and CYP2A6*3 alleles were found in a tobacco-dependent group as compared to a never tobacco-dependent group, and that smokers carrying an inactive CYP2A6 allele smoked fewer cigarettes. These findings have, however, been questioned. First, the original nonspecific genotyping method (Fernandez-Salguero et al., 1995) was used, and a total allele frequency of 6 to 10% was found for the CYP2A6*2 and CYP2A6*3 alleles. As discussed above, this genotyping method leads to a significant overestimation of the CYP2A6*2 allele frequency, and it also scores many individuals as positive for the CYP2A6*3 allele, an allele which does not seem to exist. Second, several groups have failed to reproduce the correlation between CYP2A6 genotype and smoking behavior using both the original as well as more specific genotyping methods (London et al., 1999; Sabol and Hamer, 1999; Loriot et al., 2001; Tiihonen et al., 2000). It would be interesting to conduct this kind of study in Asian populations where the frequency of inactive alleles is considerably higher and the impact on interindividual variability in nicotine metabolism should be much greater. Furthermore, it would be beneficial to phenotype the individuals with coumarin as an indicator of in vivo CYP2A6 activity, since
there are probably still a number of CYP2A6 alleles that have not yet been identified. This would clarify the predictive value of CYP2A6 genotyping for the alleles known presently. It should also be remembered that nicotine addiction and smoking behavior are complex multifactorial processes that involve many different molecular targets. It is not therefore surprising that a number of other candidate genes have been proposed as modulators of smoking behavior, including genes involved in dopamine biosynthesis and metabolism, as well as dopamine receptors, nicotinic acetylcholinergic receptors, and other enzymes involved in nicotine metabolism (Rossing, 1998).

Implications of the CYP2A6 Polymorphism for Lung Cancer Susceptibility

Most human cancers are the result of an interaction between the environment and the individual’s genetic predisposition (Perera, 1997). In the case of lung cancer, the individual’s pattern of enzymes, which activate and inactivate the precarcinogens inhaled in cigarette smoke, could modulate the risk of cancer development. The CYP2A6 genotype is one of the polymorphisms that has been suggested to modulate this risk, but as the progression of a normal cell into a tumor is a complex multistep process this is probably modulated by polymorphisms in a number of different genes. Theoretically, the absence of CYP2A6 enzyme could reduce the risk of lung cancer in two ways. First, if the hypothesis raised by Pianezza and coworkers (1998) is correct, individuals lacking CYP2A6 activity would not become smokers, or at least smoke fewer cigarettes. Second, as a number of precarcinogens found in tobacco smoke such as NNK, NDEA, and nitrosodiethylamine in mice and humans. Mol Carcinogen 7:268–275.

CYP2A6 and nicotine metabolism (Rossing, 1998).

Conclusions and Future Directions

Recent studies of the CYP2A6 polymorphism have revealed several novel alleles, and reliable genotyping methods are now available for the prediction of CYP2A6 PMs. Although several new CYP2A6 polymorphisms might be described in the future, CYP2A6 phenotyping studies using coumarin suggest that the most common inactive alleles, at least in Caucasian populations, have been found. It is, however, likely that there are additional alleles that modulate CYP2A6 activity and thereby nicotine metabolism, and additional studies are needed to understand the mechanisms behind the tremendous variability within the extensive metabolizer group. The involvement of the CYP2A6 polymorphism concerning interindividual differences in smoking behavior and risk of lung cancer is still very unclear. Several well-designed studies are needed to fully clarify this important issue. Furthermore, it remains to be determined how critical the CYP2A6 polymorphism is in determining an individual’s disposition of drugs that are primarily metabolized by this enzyme.

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References


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