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Review

Genetically determined metabolism of nicotine and its clinical significance

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Enzymes of the cytochrome P-450 (CYP 450) which belong to the family of oxidase enzymes, are present in cells of all organisms and play a major role in the first phase of xenobiotic metabolism. There are several isoenzymes of CYP 450 that show differences in the speed of metabolism: poor-, extensive- and ultra-rapid. Nicotine undergoes biotransformation in the liver mainly by the CYP2A6 isoform of CYP 450. There are many polymorphic isoforms of CYP2A6 affecting the metabolism of nicotine. There are also several CYP2A6 activity inhibitors and inducers among commonly used drugs. The ability of CYP2A6 isozymes to activate certain procancerogenic substances present in cigarette smoke makes their polymorphism more significant. Moreover, some isoforms may have also influence on the risk of lung cancer development by affecting the enzymatic activation of tobacco-specific nitrosamines. Metabolism of nicotine, mainly through CYP2A6, has also many clinical implications, such as efficacy and safety of the nicotine replacement therapy (NRT) or occurrence of several diseases. In summary, type of the nicotine metabolism may be a potential predictor of the clinical outcomes in patients with cardiovascular disease, addicted to nicotine and in those using NRT. The purpose of this work is to summarize current knowledge on variation in genetically determined metabolism of nicotine and its clinical significance.

Key words: nicotine, cytochrome P-450, nicotine metabolism, clinical significance

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Abbreviations: CYP 450, cytochrome P-450; NRT, nicotine replacement therapy; PM, poor metbolizer; EM, extensive metabolizer; UM, ultrarapid metabolizer; FMO3, flavin-containing monooxygenase 3; UGT, glucuronyltransferases; NMR, nicotine metabolite ratio; PXR, pregnane X receptors; TSNAs, tobacco specific nitrosamines; NF-κB, nuclear factor kappa B; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; COPD, chronic obstructive pulmonary disease

CYTOCHROME P450 ENZYMES

Cytochrome P-450 (CYP 450) enzymes belong to the family of oxidase enzymes. They are present in cells of all organisms, including humans, and play a major role in phase I of xenobiotic metabolism, as well as in the metabolism of certain endogenous substances, such as steroid hormones. The catalytic activity of CYP 450 includes a number of different reactions. These are mainly oxidations (C-, N- and S-oxidations) and dealkylations (O-, S- and N-dealkylations). The core element of the cytochrome activity is the electron transport chain in which crucial role plays the human NADPH-cytochrome P-450 oxidoreductase (Purnapatre *et al*., 2008; Di *et al*., 2009). The primary structure of the enzyme protein determines different isoenzymes of CYP 450. These isoforms of the enzyme have been classified into families and subfamilies. In human body, CYP1, CYP2, and CYP3 are involved in drug metabolism and biotransformation of xenobiotics (Purnaparte *et al*., 2008).

There are three CYP2A genes in humans (CYP2A6, CYP2A7, and CYP2A13) and one pseudogene (CYP2A18). CYP2A6 expression is mainly found in the liver, but its protein or mRNA is also expressed in other tissues (Godoy *et al*., 2002; Ding & Kaminsky, 2003). CYP2A6 is responsible for the critical step in conversion of nicotine to cotinine in human liver. CYP2A6 is also responsible for further conversion of cotinine to trans-3'-hydroxycotinine (Nakajima *et al*., 1996).

Different metabolic groups have been characterized due to genetic differences in the speed of metabolism, including poor- (PM), extensive- (EM) and ultra-rapid metabolizers (UM). The activity of CYP2A6 enzyme may be altered by genetic and environmental factors. Modification of CYP2A6 activity by drugs is a very important issue in medical and pharmaceutical practice (Di *et al*., 2009). The majority of cytochrome P-450 enzymes is present in the liver, mostly in the endoplasmic reticulum of hepatocytes. However, in much smaller amounts, they are also present in cells of other organs, such as the brain, kidneys, lungs, heart, and even skin.

The amount of CYPs in the brain parallels approximately 0.5–2% of that in the liver and is too low to significantly influence the overall pharmacokinetics of drugs and hormones in the body, but can play a role in modulating sensitivity to exogenous and endogenous compounds. Distribution of CYPs in the human brain varies among different brain regions. Within cortex, CYP2B6 is highly expressed in astrocytes surrounding cerebral blood vessels, whereas CYP2D6 is highly found in pyramidal neurons and also in the white matter. There are also differences between smokers and non-smokers. In the cerebellum of non-smokers, CYP2B6 and CYP2D6 are expressed in neurons within the molecular and granular layers, but are undetectable in Purkinje cells, however, in smokers, CYP2B6 and CYP2D6

are highly expressed in the Purkinje cells of the cerebellum (Miksys *et al.*, 2003; Howard *et al.*, 2003).

Smokers and alcoholics have higher levels of CYP2B6, CYP2E1, and CYP2D6 in specific brain regions. They may respond differently to certain drugs and toxins due to the elevated levels of CYPs in the brain. CYP2B6, which can also metabolize nicotine, is expressed in the brain. The CYP2B6 gene is polymorphic and smokers who are CYP2B6 slow metabolizers have increased withdrawal symptoms, higher craving and lower quitting rates (Lee *et al.*, 2007; Lerman *et al.*, 2002).

DRUGS METABOLISM

Cytochrome P-450 enzymes are responsible for the metabolism of about 78% of drugs. The most significant CYP 450 isoforms are: CYP3A4 (50% of drugs), CYP2D6 (30% of drugs), CYP2C9 (10% of drugs), CYP1A2 (4% of drugs), CYP2A6 (2% of drugs), CYP2C19 (2% of drugs), and CYP2E1 (2% of drugs) (Guengerich, 2006). Other groups are mainly responsible for the metabolism of endogenous substances and ensure the proper functioning of the cell (Purnapatre *et al.*, 2008). CYP3A subfamily, representing nearly 30% of liver- and 70% of intestinal CYP 450 enzymes, is responsible for biotransformation of more than 50% of medicines (Zuber *et al.*, 2002). In contrast, CYP2D6 isoform is responsible for the metabolism of 25–30% clinically used drugs (Ma *et al.*, 2002).

METABOLISM OF NICOTINE

Nicotine is metabolized in the liver to six major metabolites (Fig. 1). The major metabolite is cotinine, to which about 70–80% of the absorbed nicotine is metabolized. This process consists of two stages. The first step is the formation of nicotine-Δ1'(5')-iminium ion, which is in balance with the 5'-hydroxynicotine. This step is catalyzed by CYP2A6 isoform. The second step involves oxidation of nicotine-Δ1' (5')-iminium ion to cotinine under the influence of cytoplasmic aldehyde oxidase (Benowitz *et al.*, 2009). A small amount of nicotine (4–7%) is metabolized by flavin-containing monooxygenase 3 (FMO3) to another significant metabolite, nicotine N'-oxide. The nicotine N'-oxide does not undergo significant changes, except reduction to nicotine in the intestinal epithelium. In addition to oxidation reactions, nicotine is metabolized by glucuronidation to form N-beta- (S) nicotine glucuronide. The last reaction is catalyzed by glucuronyltransferases (UGT). 3–5% of nicotine is converted in this reaction. A final important metabolite directly formed from nicotine through oxidative N-demethylation is nornicotine (Hukkanen *et al.*, 2005). As much as 70–80% of nicotine is metabolized to cotinine in humans, but only 10–15% is excreted as free cotinine in urine. The major metabolites of cotinine are cotinine glucuronide and trans-3'-hydroxycotinine (Crawford *et al.*, 1998; Raunio *et al.*, 2001; Benowitz *et al.*, 2009). Also, other CYP 450 isoforms, such as CYP2B6, CYP2D6, and CYP2E1, are involved in the metabolism

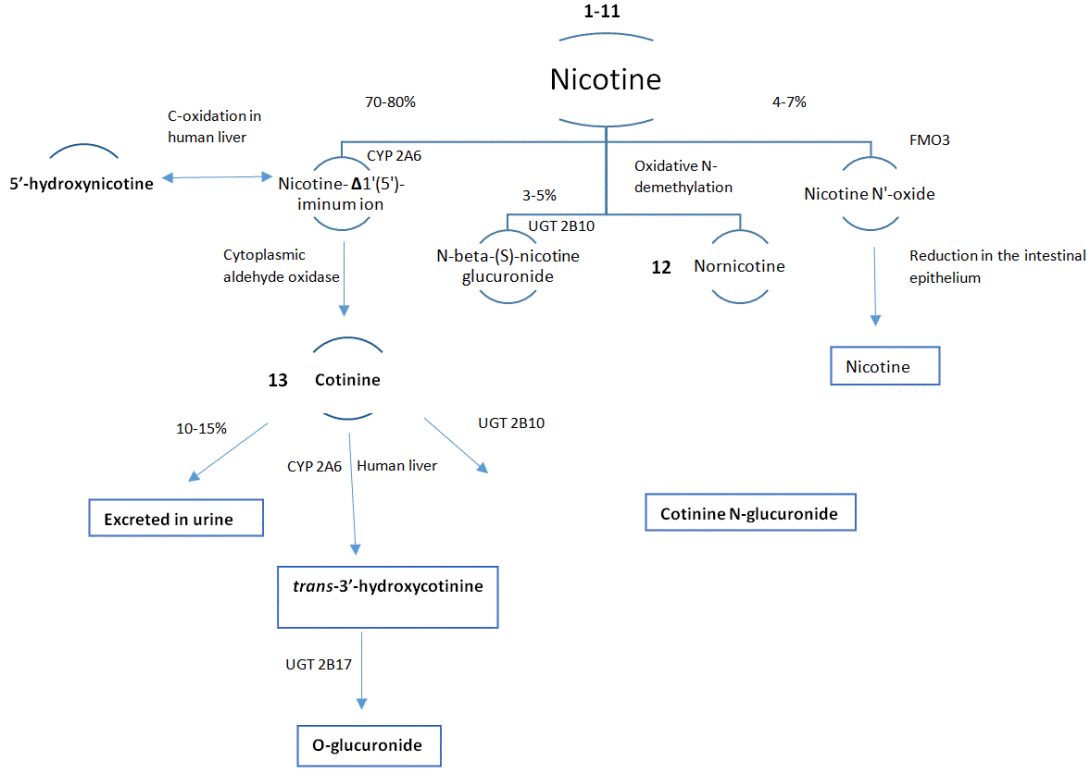

Figure 1. Metabolism of nicotine and its clinical significance (Cashman *et al.*, 1992; Berkman *et al.*, 1995; Nakajima *et al.*, 1996; Crawford *et al.*, 1998; Raunio *et al.*, 2001; Dempsey *et al.*, 2004; Hukkanen *et al.*, 2005; Benowitz *et al.*, 2009; Kosmider *et al.*, 2017; Green *et al.*, 2002; Vezina *et al.*, 2007; Dani *et al.*, 1996; IARC 2004; Moran *et al.*, 2012).
1, increase in pulse rate and blood pressure; 2, increase in plasma free fatty acids and the level of catecholamines in the blood; 3, desensitization of the GABAergic neurons by chronic stimulation by nicotine (addiction); 4, insulin resistance and predisposition to metabolic syndrome; 5, activation of signal transduction pathways, allowing the survival of damaged epithelial cells; 6, precursor of tobacco specific nitrosamines (TSNAs) through nitrosation; 7, substrate for arachidonic acid metabolites which cause increased cell division; 8, acceleration of tumor growth by increased angiogenesis mediated by β-adrenergic activation; 9, activation of nuclear factor kappa B (NF-kB)-dependent survival of cancer cell and proliferation; 10, promotion of the growth of lung tumor by inhibiting anti-apoptotic pathway; 11, cause of resistance to the chemotherapeutic agents; 12, plays a contributory role in tobacco dependence; 13, modulation of the nAChR sensitivity to agonists.

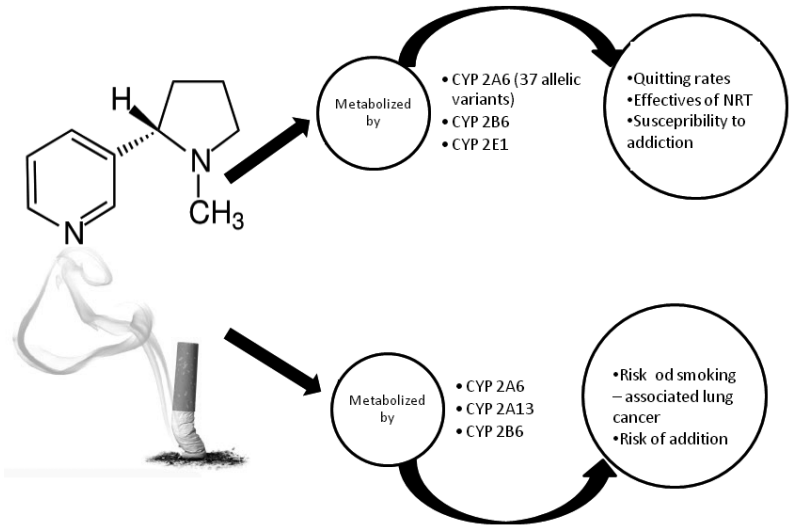

Figure 2. Similarity and differences in the metabolism of nicotine and cigarette smoke (Wong H *et al*., 2005; Su *et al*., 2000).

of nicotine (Hukkanen *et al*., 2005). Both, *in vitro* and *in vivo* studies indicate that CYP2A6 is mainly responsible for oxidation of nicotine to cotinine. In studies on the activity of microsomal enzymes, cotinine formation from nicotine and trans-3'-hydroxycotinine formation from cotinine, were correlated with the activity of coumarin 7-hydroxylase, which was strictly related to the activity of CYP2A6 (Cashman *et al*., 1992; Berkman *et al*., 1995; Nakajima *et al*., 1996). The ratio of the concentrations of 3-hydroxycotinine to cotinine in plasma and saliva is known as the nicotine metabolite ratio (NMR). This ratio is widely used for phenotyping CYP2A6 metabolic activity thanks to long half-life of cotinine (Dempsey *et al*., 2004). This non-invasive method, enabling to assess CYP2A6 activity, may be helpful in predicting the nicotine intake (Ross *et al*., 2016) and the effectiveness of smoking cessation therapies, like NRT (Chen *et al*., 2013).

DRUGS AFFECTING CYP2A6 ACTIVITY

CYP2A6 activity can be modified by drugs. There are several inhibitors, as well as inducers, of CYP2A6 among medicaments. It has been shown that 8-methoxypsoralen (a furanocoumarin used in the treatment of psoriasis, vitiligo and cutaneous T-cell lymphoma) is a potent, irreversible CYP2A6 inhibitor *in vitro* (Koenigs *et al*., 1997) and moderately effective human CYP2A6 inhibitor *in vivo* (Kharasch *et al*., 2000). Pilocarpine, a natural cholinomimetic alkaloid derived from *Pilocarpus jaborandi*, inhibits CYP2A6 and other enzymes involved in drug metabolism in mice and humans (Kimonen *et al*., 1995). Tranylcypromine, a nonselective monoaminooxidase inhibitor for the treatment of depression, is a selective potent CYP2A6 inhibitor (Taavitsainen *et al*., 2001). Also, selegiline, a selective monoaminooxidase B inhibitor used in the treatment of Parkinson's disease, was shown to be a CYP2A6 inhibitor (Siu & Tyndale, 2008). Antifungal drugs, such as klotrimazole, ketoconazole, and mikonazole are strong inhibitors of the CYP2A6 activity (Draper *et al*., 1997). Dexamethasone induces CYP2A6 activity by facilitating HNF4α (hepatic nuclear factor 4α) binding to HNF4α-RE (HNF4α response element) which increases the *CYP2A6* transcriptional activity. This may be caused by changed histone H4 acetylation in proximal promoter of the *CYP2A6* gene (Onica *et al*., 2008). Rifampin and phenobarbital also belong to CYP2A6 inducers (Maurice *at al*., 1991; Rae *et al*., 2001). Interestingly, atorvastatin, fluvastatin, and rosuvastatin are associated with activation of pregnane X receptors (PXR) and increase in the CYP2A6 activity. A degree of this effect depends on statin and its enantiomer (Korhonova *et al*., 2015).

GENETIC POLYMORPHISM OF CYP2A6

There are more than 37 different allelic variants of CYP2A6 (Gaedigk *et al*., 2018). Studies have shown that among homozygous individuals with the *CYP2A6* gene, there is a lack of detectable cotinine level in the plasma (Nakajima *et al*., 2000; Swan *et al*., 2009). Wild type refers to *CYP2A6* 1A*. Polymorphic variants of this enzyme's gene may cause alterations in enzymatic activity of the encoded enzyme. Each allele accompanied with another allele can form a homozygote or heterozygote of the *CYP2A6* locus. *CYP2A6*2* goes together with *CYP2A6*2* composing homozygote with slow metabolism. Similarly, *CYP2A6*2* and *CYP2A6*4* form a "poor metabolizer" (PM) heterozygote and both) also form a PM heterozygote when present together with *CYP2A6*1* (Benowitz *et al*., 2006). *CYP2A2*1* determines rapid metabolism of nicotine, whereas CYP2A6 alleles **2* and **4* are responsible for the reduced metabolism ratio. Their occurrence is closely associated with the ethnicity. Alleles **2* and **4* are present in 1% of Caucasian and 20% of Japanese populations, respectively (Nakajima *et al*., 2006). *In vivo* and *in vitro* studies on the *CYP2A6*2* variant had shown that this allele determines the total lack of activity of the encoded enzyme (Fernandez-Salguero *et al*., 1995). *CYP2A6*4* with total deletion of the gene has no enzymatic activity (Oscarson *et al*., 1999). Variants associated with a single nucleotide polymorphism (SNP) are: *CYP2A6*2, CYP2A6*5, *6 CYP2A6 , CYP2A6, CYP2A6 *7* and **9* (O'Loughlin *et al*., 2004). *CYP2A6*10* has reduced enzymatic activity (Yoshida *et al*., 2002). The same polymorphism could have a different effect on different substrates, thus it should be examined in regard to the individual substrates (Tyndale & Sellers, 2002). The differences also apply to medicines used in cancer treatment, such as letrozole. CYP2A6*12 heterozygote individuals are intermediate nicotine metabolizers and simultaneously slow letrozole metabolizers (Fukami *et al*., 2005).

FREQUENCY OF CYP2A6 ISOFORMS IN THE HUMAN POPULATION

Proportion of people with slow nicotine metabolism by CYP2A6 (activity less than 50%) ranges from about 10% in the Caucasian population to approximately 60% in the Japanese population (Fukami *et al*., 2005). The frequency of *CYP2A6* alleles determining slow type of metabolism, such as: *CYP2A6*4, *7* and **9,* is much higher among Chinese (7%, 3%, 16%, respectively), Japanese (17%, 11%, 20%, respectively), and Korean populations (11%, 4%, 22%, respectively) in comparison to Caucasians, where frequency of these variants is extremely rare (1 and 0% for alleles **4* and **7,* respectively). The incidence of **9* in the Swedish population is at 5%, and it is at 7% in the Turkish population (Minematsu *et al*., 2006).

POTENTIAL ROLE OF CYTOCHROME P-450 POLYMORPHISM IN CARCINOGENESIS

The ability of CYP2A6 enzymes to activate certain procarcinogens present in the cigarette smoke makes their polymorphism more significant. The complexity of carcinogens and compounds present in tobacco smoke results in an indirect link between a particular carcinogen and specific tumor (Rossini *et al*., 2008).

The overlapping substrate specificity between CYP2A6 and CYP2A13 results in the involvement of these two enzymes in the metabolism of tobacco constituents. CYP2A13 has a higher catalytic efficiency towards 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 2'-hydroxylation of N-nitrosonornicotine and expression of this enzyme varies between different tissues (Wong H *et al*., 2005; Su *et al*., 2000) (Fig. 2).

Hecht and others (Hecht *et al*., 2000) reported a new pathway of 2'-hydroxylation of nicotine *via* 2'-hydroxynicotine, which leads to the formation of 4-(methylamino)-1-(3-pyridyl)-1-butanone. This compound can be converted *in vitro* into a carcinogenic nitrosamine. However, endogenous production of NNK in the human body has not yet been established. Taking into consideration the ability of CYP2A6 metabolism to generate nitrosamines, London and others (London *et al*., 1999) hypothesized that individuals with slower metabolism may have a lower risk of smoking-associated lung cancer. Lower rate of nicotine metabolism by CYP 450 enzymes is correlated with a lower risk of addiction to nicotine. According to Tyndale and others (Tyndale *et al*., 2002), individuals with alleles responsible for defective nicotine metabolism had a decreased risk of becoming a smoker or they smoked less in comparison to extensive nicotine metabolizers. Slower metabolizers may also activate less tobacco-related procarcinogens in comparison to extensive metabolizers. The type of metabolism also affects nicotine obtained from NRT. Also, the *CYP2B6* polymorphism has influence on the risk of lung cancer development by affecting enzymatic activation of tobacco -specific nitrosamines, both, in conjunction with and regardless of the activity of the CYP2A6 enzyme (Wassenaar *et al*., 2013).

IMPLICATIONS FOR SMOKING CESSATION THERAPY

Smokers that have alleles associated with high activity of the enzyme (*CYP2A6*1/*1B*) experience serious symptoms of abstinence during breaking the habit. This correlation is more visible in NRT- using individuals when the delivered dose of nicotine is reduced with the duration of therapy (Kubota *et al*., 2006). People with a low rate of nicotine metabolism smoke less cigarettes and therefore need lower doses of nicotine from NRT (O'Loughlin *et al*., 2004). Individuals with normal enzyme activity become more easily addicted than those with a lower rate of metabolism and expressing *CYP2A6*9, CYP2A6*2 or CYP2A6*4* alleles (Audrain-McGovern *et al*., 2007). According to Lerman and others (Lerman *et al*., 2006), in the group using transdermal nicotine, the intensity of cravings increased linearly with the increased NMR. It has been also shown that drugs that inhibit the function of the CYP2A6 reduce the number of cigarettes smoked per day (Sellers *et al*., 2000). Methoxsalen

Table 1. Clinical implications related to the type of nicotine metabolism.

CYP 450 isoforms	Alleles	Type of nicotine metabolism	Clinical significance	Source
CYP2A6	CYP2A6*2	poor	associated with more likely quitting of smoking	(Gu *et al*., 2000)
CYP2A6	*CYP2A6*7 CYP2A6*4 CYP2A6*10*	poor	the impairment of the enzyme is associated with protection from hepatoxic effect of drugs, such as valproic acid	(Sadeque *et al*., 1997)
CYP2A6	CYP2A6*3	poor	associated with protective role against nicotine addiction	(Oscarson *et al*., 1999)
CYP2A6	CYP2A6*1	extensive	wild type of gene is associated with higher risk of lung cancer, as it was proved to be more frequent in cancer patients than in control group	(Miyamoto *et al*., 1999)
CYP2A6	CYP2A6*4C	poor	unable to activate nicotine-derived carcinogens, N-nitrosamines such as NNK, which results in lower risk of cancer	(Miyamoto *et al*., 1999)
CYP2A6	CYP2A6*1B	ultrarapid	higher NMR in comparison to wildtype homozygotes. Association with smoking behaviour and response to NRT. Increased likelihood of being a smoker and higher amount of cigarettes smoked per day	(Gambier *et al*., 2005)
CYP2A6	CYP2A6*7	poor	lower cigarette consumption per day, shorter smoking duration	(Liu *et al*., 2011)
CYP2A6	CYP2A6*17	poor	increased ability to quit smoking habit	(Ho *et al*., 2009)
CYP2A6	CYP2A6*35	poor	increased ability to quit smoking habit	(Al Koudsi *et al*., 2009)

is an example of CYP2A6 inhibitor (Damaj *et al*., 2007). In pharmacokinetic studies on methoxalen, after its oral administration post an overnight period of abstinence, higher concentrations of nicotine in the plasma were observed. A reduced desire for smoking was also observed (Sellers & Tyndale, 2000). Therefore, it is thought that inhibition of the CYP2A6 activity could be considered as a smoking cessation strategy. The polymorphism of *CYP2A6* is also connected with the age of smoking initiation and the age of occurrence of chronic obstructive pulmonary disease (COPD) in smoking patients (Siedlinski *et al*., 2011). Inhibition of CYP2A6 could enhance the effectiveness of nicotine gums, patches and other forms of NRT, due the fact that these forms provide about 50% of the plasma concentration obtained by smoking (Sellers *et al*., 2003; Benowitz *et al*., 1997).

CLINICAL IMPLICATIONS OF NICOTINE METABOLISM

Metabolism of nicotine, mainly through CYP2A6, also has many clinical implications, including efficacy and safety of NRT and occurrence of several diseases. This makes the type of nicotine metabolism a potential predictor of clinical outcomes in patients with cardiovascular disease, addicted to nicotine, and those using NRT, as well as taking other medications. The most significant clinical implications related to the type of nicotine metabolism are summarized in Table 1.

CONCLUSIONS

The variety of cytochrome P-450 isoforms that take part in nicotine metabolism makes this topic significant due to its clinical consequences. In reference to CYP2A6, likelihood of becoming a smoker, consumption of cigarettes per day, and response to nicotine replacement therapy are often mentioned in contrast to other processes, such as metabolism of drugs and carcinogenesis. Genotyping toward CYP2A6 may be a helpful clinical tool in a group of patients using nicotine, irrespectively of the way of its administration, in order to assess their type of nicotine metabolism and its clinical significance.

Conflicts of interest

LK works as an expert for the Polish National Committee for Standardization and for the European Committee for standardization of requirements and test methods for e-liquids and emissions. LK was also an employee of the Institute of Occupational Medicine and Environmental Health. One of the institute's objectives is outsourcing for the industrial sector, including manufacturers of e-cigarettes. However, this has no influence on the study's design.

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