Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 77 (2004) 793–795
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Polymorphisms of glutathione S-transferases M1 and T1 do not account for interindividual differences for smoking behavior

Mostafa Saadat*, Hassan Mohabatkar

Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran

Received 5 November 2003; received in revised form 2 February 2004; accepted 10 February 2004

Abstract

To identify whether the polymorphisms of glutathione S-transferase M1 (GSTM1) and GSTT1 genes predict a high-tended risk of using tobacco, the GSTM1 and GSTT1 genotypes of 369 Iranian males (254 nonsmokers and 115 smokers) and 314 Iranian females (245 nonsmokers and 69 smokers) were determined. The frequencies of GSTM1 (males: OR = 0.98, 95% CI = 0.62–1.57, *P*=.974; females: OR = 1.34, 95% CI = 0.75–2.39, *P*=.358) and GSTT1 (males: OR = 1.25, 95% CI = 0.76–2.04, *P*=.412; females: OR = 0.84, 95% CI = 0.46–1.51, *P*=.626) null genotypes were similar in nonsmokers and smokers. The risk of being a smoker was to be equally frequent in each combination of the genotypes. The present results revealed that there was no difference between smokers and nonsmokers for these two genetic polymorphisms.

Keywords: Cigarette smoking; Genetic polymorphisms; Glutathione S-transferase

1. Introduction

Genetic studies suggest that all stages of tobacco use and dependence, maintenance of dependent smoking behavior, and amount smoked are partially under genetic control (Hall et al., 2002). The study of smoking behavior in adult adoptees and their biological and adoptee family supports the finding in twin studies of a genetic influence on smoking within the same generation (Osler et al., 2001).

Cigarette smoke contains several thousand various compounds. Many of these compounds are substrates of Phase I enzymes, represented by cytochrome P450 enzymes (Nakajima et al., 2001; Tyndale and Sellers, 2002; Yang et al., 2001). Cytochrome P450 2A6 (CYP2A6) has been shown to activate a variety of precarcinogens such as 4-(methylnitros-amino)-1-(3-pyridyl)-1-butanone, *N*-nitrosodiethylamine, afaltoxin B1, 4,4′ -methylene-bis (2-chloroaniline), 1,3-butadiene 2,6-dichlorobenzonitrile, and nicotine. In addition, CYP2A6 is involved in the metabolism of certain pharmaceuticals such as methoxyflurane and halothane (Oscarson et al., 1998). The genetically polymorphic CYP2A6 enzyme is responsible for the majority of the metabolism of nicotine (Tyndale and Sellers, 2002; Yang et al., 2001). There is some evidence that CYP2A6 poor-metabolizer genotypes result in altered nicotine kinetics (Pianezza et al., 1998; Tyndale and Sellers, 2002). In addition, it is reported that the CYP2D6 ultrarapid metabolizer genotype may contribute to the probability of being addicted to smoking (Saarikoski et al., 2000).

Glutathione S-transferases (GSTs) are one of the most important Phase II enzymes involved in detoxification. The GSTs are involved in the detoxification of many toxic compounds of different chemical structures in cigarette smoke, including epoxybutanes, ethylene oxide, monohalomethane, reactive metabolites of polycyclic aromatic hydrocarbons such as benzo[a]pyrene (Ketterer et al., 1992; Meyer et al., 1991; Rebbeck, 1997). There are well-defined genetic polymorphisms in the expression of glutathione S-transferase M1(GSTM1) and GSTT1 enzymes with non-functional null alleles named GSTM1-0 and GSTT1-0. The homozygosity for these alleles associated with the absence of the corresponding enzyme activity (Harada et al., 1992; Pemble et al., 1994).

Cancer and atherosclerosis share common risk factors such as cigarette smoking. The GSTM1 and GSTT1 null

* Corresponding author. Tel.: +98-711-2282747; fax: +98-711-2280926.
E-mail address: saadat@susc.ac.ir (M. Saadat).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.02.003

genotypes have been linked with an increased risk of several types of cancer including lung cancer (Harada et al., 1992; Nakachi et al., 1993; Saadat and Saadat, 2001). Because GSTM1 and GSTT1 are present in the human lung tissue (Antilla et al., 1995; Mainwaring et al., 1996), these enzymes may play important roles on the lung functions. In addition, some reports suggested that polymorphisms of GSTM1 and GSTT1 are associated with the risk of atherosclerosis (Li et al., 2000; Salama et al., 2002).

Despite increasing scientific evidence for a causal role of tobacco smoking in human cancers and coronary heart disease, an alternative hypothesis was put forward. The constitutional hypothesis has stated that there are genetic or other common factors that predispose both to smoking and disease. Therefore, to identify whether the polymorphisms of GSTM1 and GSTT1 genes predict a high-tended risk of using tobacco, the present study was done.

2. Material and methods

2.1. Subjects

Blood samples from 369 Iranian males (254 nonsmokers and 115 smokers) and 314 Iranian females (245 nonsmokers and 69 smokers) were collected. Nonsmoker means that a person has no history of smoking. Informed consent was obtained from all participants at the time of blood withdrawal. The mean age of the subjects was 39.7 ± 10.4 (S.D.) years.

2.2. Genotyping

Genomic DNA for PCR was isolated from the whole blood samples. The assay for GSTM1 and GSTT1 genotyping has been described previously (Saadat and Saadat, 2001). A 1030-bp fragment was amplified by PCR with the GSTM1 primers, and a 480-bp fragment was amplified by PCR with the GSTT1 primers. The absence of an amplified product was consistent with the null genotypes (Harada et al., 1992; Pemble et al., 1994). The human β-globin gene was also amplified in each reaction as a positive control to confirm the presence of amplifiable DNA in the sample. The negative controls (tubes containing the PCR mixture without the DNA template) were included in every run. Two independent readers interpreted the gel photographs. A random selection of 15% of all samples was repeated. No discrepancy was discovered upon replicate testing.

2.3. Statistical analysis

The relative associations between the genotypes and smoking status were assessed by calculating the odds ratios (OR) and 95% confidence intervals (CIs). A P value less than .05 was considered to indicate significance.

3. Results and discussion

The frequencies of GSTM1 and GSTT1 null genotype in nonsmokers and smokers of males and females are shown in Table 1. The statistical analysis of the frequencies of GSTM1 (males: OR = 0.98, 95% CI = 0.62–1.57, P=.974; females: OR = 1.34, 95% CI = 0.75–2.39, P=.358) and GSTT1 (males: OR = 1.25, 95% CI = 0.76–2.04, P=.412; females: OR = 0.84, 95% CI = 0.46–1.51, P=.626) null genotypes was similar in nonsmokers and smokers.

It has already been shown that GSTM1 and GSTT1 are involved in detoxification of a variety of compounds, some of which overlap between these enzymes and some which are highly specific (Hayes and Pulford, 1995). In the present study, we have examined the risk of being a smoker associated with combinations of the genotypes. The reference group consisted of individuals with two putative low-tended genotypes, i.e., the presence of GSTM1 and GSTT1 functional genotype alleles. The risk of being a smoker was to be equally frequent in each combination of genotypes in both sexes (Table 2). The Chi-square for trend in risk associated with zero, one, and two putative high-tended genotypes was not statistically significant (Males: $\chi^2 = 0.350$, P=.554; females: $\chi^2 = 0.092$, P=.759; see Table 2).

Individuals lacking full function CYP2A6 alleles do not metabolize nicotine and are less likely to become smokers, and if they do, they smoke fewer cigarettes per day in comparison with normal-nicotine metabolism persons (Pianezza et al., 1998; Tyndale and Sellers, 2002). The CYP2E1 and CYP2D6 polymorphisms may induce individual variability in smoking cessation or nicotine addiction (Saarikoski et al., 2000; Yang et al., 2001). However, in some reports, other cytochrome P-450s, such as CYP1A1 and CYP2D6, did not show association with the smoking phenotype (Caporaso et al., 2001; Yang et al., 2001). Because genetic polymorphisms of Phase I enzymes associated with the smoking phenotype, it was speculated that the polymorphism of the Phase II enzyme also correlated

Table 1
Association between studied genotypes and smoking status

Genotypes	Nonsmokers	Smokers	OR	95% CI	P value
Males					
GSTM1					
Present	138	63	1.0	(Reference)	
Null	116	52	0.98	(0.62–1.57)	.974
GSTT1					
Present	176	74	1.0	(Reference)	
Null	78	41	1.25	(0.76–2.04)	.412
Females					
GSTM1					
Present	117	28	1.0	(Reference)	
Null	128	41	1.34	(0.75–2.39)	.358
GSTT1					
Present	146	44	1.0	(Reference)	
Null	99	25	0.84	(0.46–1.51)	.626

Table 2
Association between the combination of the GST genotypes and smoking status

Genotypes		Nonsmokers	Smokers	OR	(95% CI)	*P* value
GSTM1	GSTT1					
Males						
Two putative, low-tended genotypes						
Present	Present	94	40	1.0	(Reference)	
One putative, high-tended genotype						
Present	Null	44	23	1.23	(0.63–2.40)	.629
Null	Present	82	34	0.97	(0.54–1.74)	.694
Total		126	57	1.06	(0.64–1.78)	.901
Two putative, high-tended genotypes						
Null	Null	34	18	1.24	(0.60–2.59)	.650
Females						
Two putative, low-tended genotypes						
Present	Present	73	19	1.0	(Reference)	
One putative, high-tended genotype						
Present	Null	44	9	0.79	(0.30–2.04)	.748
Null	Present	73	25	1.32	(0.63–2.74)	.534
Total		117	34	1.12	(0.57–2.21)	.856
Two putative, high-tended genotypes						
Null	Null	55	16	1.12	(0.49–2.53)	.922

The χ^2 for the trend of none, one, and two putative, high-tended genotypes in males and females were 0.350 (P=.554) and 0.092 (P=.759), respectively.

with the phenotype. Our data, however, failed to show an association between cigarette smoking and polymorphisms of GSTM1 and GSTT1.

Acknowledgements

We are indebted to Dr. Maryam Ansari-Lari for critical reading of the manuscript, Mr. Iraj Saadat for his contribution in discussion, and to the participants for their close cooperation. This study was supported by Shiraz University.

References

Antilla A, Luostariner L, Hirvonen A, Elovaara E, Karjalainen A, Nurminen T, et al. Pulmonary expression of glutathione S-transferase M3 in lung cancer patients: association with GSTM1 polymorphism, smoking, and asbestos. Cancer Res 1995;55:3305–9.

Caporaso EN, Lerman C, Audrain J, Boyd NR, Main D, Issaq HJ, et al. Nicotine metabolism and CYP2D6 phenotype in smokers. Cancer Epidemiol. Biomarkers Prev 2001;10:61–3.

Hall W, Madden P, Lynskey M. The genetics of tobacco use: methods, findings and policy implications. Tob Control 2002;11:119–24.

Harada S, Misawa S, Nakamura T, Tanaka N, Ueno E, Nozoe M. Detection of GST1 gene deletion by the polymerase chain reaction and its possible correlation with stomach cancer in Japanese. Hum Genet 1992;90:62–4.

Hayes JD, Pulford DJ. The glutathione S-transferase super-gene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem 1995;30: 445–600.

Ketterer B, Harris JM, Talaska G. The human glutathione S-transferase family, its polymorphism, and its effects on susceptibility to lung cancer. Environ Health Perspect 1992;98:87–94.

Li R, Boerwinkle E, Olshan AF, Chambless LE, Pankow JS, Tyroler HA, et al. Glutathione S-transferase genotype as a susceptibility factor in smoking-related coronary heart disease. Atherosclerosis 2000;149: 451–62.

Mainwaring GW, Williams SM, Foster JR, Tugwood J, Green T. The distribution of theta-class glutathione S-transferase in the liver and lung of mouse, rat and human. Biochem J 1996;318:297–303.

Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B. Theta, a new class of glutathione transferase purified from rat and man. Biochem J 1991;274:409–14.

Nakachi N, Imai K, Hayashi SI, Kawajiri K. Polymorphism of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. Cancer Res 1993;53:2994–9.

Nakajima M, Kwon JT, Tanaka N. Relationship between interindividual differences in nicotine metabolism and CYP2A6 genetic polymorphism in humans. Clin Pharmacol Ther 2001;69:72–8.

Oscarson M, Gullsten H, Rautio A, Bernal ML, Sinues B, Dahl ML, et al. Genotyping of human cytochrome P450 2A6 (CYP2A6), a nicotine C-oxidase. FEBS Lett 1998;438:201–5.

Osler M, Holst C, Prescott E, Sorensen TIA. Influence of genes and family environment on adult smoking behavior assessed in an adoption study. Genet Epidemiol 2001;21:193–200.

Pemble S, Schroeder KR, Spencer SR, Hallier DJ, Bolt HM, Ketterer B, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 1994; 300:271–6.

Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking. Nature 1998;393:750.

Rebbeck TR. Molecular epidemiology of the human glutathiomne S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prevent 1997;6:733–43.

Saadat I, Saadat M. Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Cancer Lett 2001; 169:21–6.

Saarikoski ST, Sata F, Husgafvel-Pursiainen K, Rautalahti M, Haukka J, Impivaara O, et al. CYP2D6 ultrarapid metabolizer genotype as a potential modifier of smoking behaviour. Pharmacogenetics 2000;10:5–10.

Salama SA, Au WW, Hunter GC, Sheahan RG, Badary OA, Abdel-Naim AB, et al. Polymorphic metabolizing genes and susceptibility to atherosclerosis among cigarette smokers. Environ Mol Mutagen 2002;40: 153–60.

Tyndale RF, Sellers EM. Genetic variation in CYP2A6-mediated nicotine metabolism alters smoking behavior. Ther Drug Monit 2002;24: 163–71.

Yang M, Kunugita N, Kitagawa K, Kang S, Coles B, Kadlubar FF, et al. Individual differences in urinary cotinine levels in Japanese smokers: relation to genetic polymorphism of drug-metabolizing enzymes. Cancer Epidemiol Biomarkers Prev 2001;10:589–93.