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No Association between Polymorphisms in *CYP2E1, GSTM1, NAT1, NAT2* and the Risk of Gastric Adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition

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Introduction

Metabolizing enzymes of many procarcinogens present in tobacco smoke often display genetic polymorphisms; thereby, genetic variation of such enzymes can modulate susceptibility to gastric cancer risk. The ethanol-inducible enzyme cytochrome *P*450 2E1 (CYP2E1) metabolizes dietary and tobacco-specific nitrosamines; *N*-acetyltransferases (NAT1 and NAT2) play a role in the metabolism of aromatic amines and heterocyclic amines; finally, subjects with deletion of the phase II enzyme glutathione *S*-transferase (GST) have a decreased capability of detoxifying several carcinogens (1). Despite the biological plausibility and epidemiologic studies investigating the relationship between cancer in humans and these enzymes, there are still conflicting results regarding their etiologic relevance in gastric carcinogenesis (2). We have reported separately the association between gastric cancer risk and genetic variation in CYP1A1 and CYP1A2, microsomal epoxide hydrolase (EPHX1), and GSTT1 involved in the metabolism of polycyclic aromatic hydrocarbons. Here, we present the results concerning the relationship between polymorphisms in CYP2E1, NAT1, NAT2, and GSTM1 and the risk of gastric adenocarcinoma, as well as potential effect modification of such association by tobacco smoking.

Materials and methods

Subjects

The study subjects were selected according to a nested case-control design from the EPIC cohort, which includes about half million individuals recruited between 1992 and 1998 in 23 centers in 10 European countries (3). Cases were subjects newly diagnosed during the follow-up of gastric adenocarcinoma, confirmed and validated by an independent panel of pathologists. For each case, up to four controls were randomly selected among cohort members alive and free of cancer at the time of diagnosis of the case, with blood samples available, matched by gender, age, center, and date of blood collection. The final study group consisted of 243 cases and 946 matched controls.

Genotyping

Genomic DNA was extracted from a 0.5-mL aliquot of buffy coat as reported.[34] Single nucleotide polymorphisms (SNP) genotyping was done in a LightCycler instrument (Roche Diagnostics, Mannheim, Germany) by melting curve analysis of a fluorescently labeled sensor probe specific for each PCR amplified variant. The four *NAT2* polymorphisms were analyzed by use of the LC NAT2 Mutation Detection kit (Roche Molecular Biochemicals, Mannheim, Germany). Primers and hybridization probes were obtained from published literature (4, 5). Genes have been named according to the HUGO Gene Nomenclature committee (http://www.genomic.unimelb.edu.au), and polymorphisms have been identified according to the SNP500Cancer database (http://snp500cancer.nci.nih.gov/home.cfm) and to the ID numbering of the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP).

Statistical Analysis

Hardy-Weinberg equilibrium for each polymorphism was tested separately for cases and controls. Association between each SNP and gastric cancer risk was assessed by the odds ratio (OR) with corresponding 95% confidence interval estimated by logistic regression (6). Effect modification by smoking status was assessed by the likelihood ratio test.

Results

In our study, 56% cases (and matched controls) were males, with average age at recruitment of 59 years. Among controls, all the genotype distributions were in Hardy-Weinberg equilibrium. The genotype distribution and ORs for all the analyzed SNPs are shown in Table 1 . No significant associations were seen when each genotype was compared with the homozygous group of the most frequent allele, or by combining heterozygotes and homozygotes for the variant allele (dominant model); in addition, no significant associations appeared carrying out the analysis according to a recessive model (data not shown). There was an increased risk in gastric cancer among ever smokers associated with the T allele of *CYP2E1* −1054C>T and the A allele of *NAT2 *7A/B*, but none of them was statistically significant (Table 2). There were no significant interactions between smoking status and any of the analyzed SNP.

Table 1. Frequency distribution of genotypes and ORs for polymorphisms in metabolic genes in gastric adenocarcinoma cases and controls

Gene	Polymorphism	Genotype	Cases, *n* (%)	Controls, *n* (%)	OR* (95% CI)
CYP2E1	−1054C>T (**5B*) rs2031920	CC	226 (94.6)	880 (95.7)	1 (reference)
		CT	13 (5.4)	39 (4.2)	1.29 (0.67-2.47)
		TT	—	1 (0.1)	—
		TT/CT	13 (5.4)	40 (4.3)	1.26 (0.66-2.41)
GSTM1	del{GSTM1}	Present	120 (49.6)	434 (46.6)	1 (reference)
		Null	122 (50.4)	498 (53.4)	0.88 (0.67-1.17)
NAT1	Ex1−88A>T (**10*) rs1057126	TT	149 (63.4)	593 (63.9)	1 (reference)
		TA	76 (32.3)	295 (31.8)	1.02 (0.75-1.40)
		AA	10 (4.3)	40 (4.3)	1.02 (0.50-2.11)
		AA/TA	86 (36.6)	335 (36.1)	1.02 (0.76-1.39)
	Ex1−81A>C (**10*) rs15561	CC	149 (63.4)	590 (63.6)	1 (reference)
		CA	72 (30.6)	288 (31.0)	0.99 (0.72-1.37)
		AA	14 (6.0)	50 (5.4)	1.14 (0.61-2.12)
		AA/CA	86 (36.6)	338 (36.4)	1.01 (0.75-1.37)

NAT2	Ex2+487C>T (*L161L, *5A*) rs1799929	CC	78 (32.5)	288 (31.2)	1 (reference)
		CT	116 (48.3)	467 (50.5)	0.91 (0.66-1.25)
		TT	46 (19.2)	169 (18.3)	1.00 (0.67-1.51)
		TT/CT	162 (67.5)	636 (68.8)	0.93 (0.69-1.26)
	Ex2–580G>A (*R197Q, *6A*) rs1799930	GG	124 (51.7)	444 (48.1)	1 (reference)
		GA	100 (41.7)	396 (42.9)	0.92 (0.68-1.24)
		AA	16 (6.7)	83 (9.0)	0.68 (0.38-1.19)
		AA/GA	116 (48.3)	479 (51.9)	0.87 (0.65-1.16)
	Ex2–313G>A (*G286E, *7A/B*) rs1799931	GG	226 (93.4)	874 (95.5)	1 (reference)
		GA	16 (6.6)	41 (4.5)	1.36 (0.75-2.49)
		AA	—	—	—
		AA/GA	16 (6.6)	41 (4.5)	1.36 (0.75-2.49)

Abbreviation: 95% CI, 95% confidence interval.

* OR estimated by conditional logistic regression.

Table 2. ORs and 95% CI of gastric adenocarcinoma for SNPs in metabolic genes according to smoking status

Gene	Polymorphism	Model	Never smokers (83 cases, 425 controls)		Ever smokers (160 cases, 521 controls)		$P_{interaction}$*
			Cases/controls†	OR‡ (95% CI)	Cases/controls†	OR‡ (95% CI)	
CYP2E1	–1054C>T (*5B*)	TT/CT vs CC	4/22	0.97 (0.31-3.05)	9/18	1.51 (0.63-3.60)	0.46
GSTM1	del{GSTM1}	Null vs present	38/223	0.79 (0.48-1.28)	84/275	1.01 (0.70-1.46)	0.45
NAT1	Ex1–88A>T (*10*)	AA/TA vs TT	30/141	1.19 (0.71-1.99)	56/194	0.92 (0.63-1.35)	0.43
	Ex1–81A>C (*10*)	AA/CA vs CC	30/141	1.19 (0.71-1.99)	56/197	0.90 (0.62-1.33)	0.39
NAT2	Ex2+487C>T (*L161L, *5A*)	TT/CT vs CC	58/290	0.95 (0.56-1.63)	104/346	0.91 (0.62-1.34)	0.87
	Ex2–580G>A	AA/GA	44/221	0.98	72/258	0.85	0.61

(*R197Q, *6A*)	vs GG		(0.60-1.59)		(0.59-1.24)	
Ex2–313G>A (*G286E, *7A/B*)	AA/GA vs GG	3/21	0.76 (0.21-2.67)	13/20	2.11 (0.99-4.50)	0.10

Abbreviation: 95% CI, 95% confidence interval.

* By likelihood ratio test.

†Number of cases and controls in the comparison category (dominant, recessive, null).

‡OR estimated by unconditional logistic regression, adjusted by sex, age, center, and date of blood extraction. For ever smokers, further adjustment by cigarettes/day and duration of smoking (y).

Discussion

CYP2E1 is involved in the metabolism of low molecular weight compounds, such as nitrosamines. Our results show that the frequency of the –1054C>T variant (allele **5B*, *Rsa*I sensitive) is lower in Caucasians than in Asian populations. Although some studies have reported lack of association with gastric cancer risk (2, 7), others observed a protective effect for the uncommon T variant (7-9), and another study reported a significant increased risk (OR, 2.9) for the homozygous variant (10). A recent meta-analysis (11) estimated an overall 24% increase in gastric cancer risk for *GSTM1* deletion, only marginally significant overall, and slightly lower in Caucasian populations. Acetylation may have substrate-specific effects: whereas it is seen as an activating mechanism for heterocyclic amines, it has detoxifying effect for aromatic amines (12). All the SNPs analyzed are associated with fast NAT1 enzymatic activity and slow acetylation by NAT2. Regarding NAT2 and gastric cancer risk, there are controversial results: two Japanese studies (8, 13) reported significant increased risks of about 60% for slow enzymatic activity, whereas a significant OR of 0.37 was observed in a study in Spain (14) and nonsignificant OR of 0.46 in the United Kingdom (15). The latter also reported a significant increased risk (OR, 2.5) for the rapid phenotype **10* in *NAT1*.

Taking into account the prevalence of the analyzed variants in our population (Table 1), we had always a power > 80% to detect an OR of >1.5 or <0.67, except for *CYP2E1*, with power < 50% because of the small frequency of variant allele T. The power was also compromised (slightly below 50%) to detect an OR of 1.24 as estimated in a meta-analysis for *GSTM1*, in spite of high proportion of deletions. Both for *NAT1* and *NAT2*, the power to detect ORs in the range of those reported in previous studies (0.4-2.5) was very high, always >90%.

In conclusion, by analyzing a large European population essentially of Caucasian origin, our study did not find any association between gastric cancer risk and several polymorphisms in genes involved in the metabolism of tobacco carcinogens. Most studies dealing with these genes have been done on Asian populations showing controversial results.

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[34] A. Agudo et al. Polymorphisms in metabolic genes related to tobacco smoke and the risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). Cancer Epidemiol Biomarkers Prev, submitted for publication.

References

1. Vineis P, Malats N, Lang M, et al., editors. Metabolic polymorphisms and susceptibility to cancer. Lyon (France): IARC Scientific Publications; 1999.

2. Gonzalez CA, Sala N, Capella G. Genetic susceptibility and gastric cancer risk. Int J Cancer 2002;100:249–60.

3. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.

4. Choi JY, Abel J, Neuhaus T, et al. Role of alcohol and genetic polymorphisms of CYP2E1 and ALDH2 in breast cancer development. Pharmacogenetics 2003;13:67–72.

5. Voso MT, D'Alo F, Putzulu R, et al. Negative prognostic value of glutathione *S*-transferase (*GSTM1* and *GSTT1*) deletions in adult acute myeloid leukemia. Blood 2002;100:2703–7.

6. Breslow NE, Day NE. Statistical methods in cancer research. Vol I: the analysis of case-control studies. IARC Scientific Publications, No. 32. Lyon (France): IARC; 1980.

7. Nishimoto IN, Hanaoka T, Sugimura H, et al. Cytochrome P450 2E1 polymorphism in gastric cancer in Brazil: case-control studies of Japanese Brazilians and non-Japanese Brazilians. Cancer Epidemiol Biomarkers Prev 2000;9:675–80.

8. Suzuki S, Muroishi Y, Nakanishi I, Oda Y. Relationship between genetic polymorphisms of drug-metabolizing enzymes (CYP1A1, CYP2E1, GSTM1, and NAT2), drinking habits, histological subtypes, and p53 gene point mutations in Japanese patients with gastric cancer. J Gastroenterol 2004;39:220–30.

9. Cai L, Zheng ZL, Zhang ZF. Cytochrome p450 2E1 polymorphisms and the risk of gastric cardia cancer. World J Gastroenterol 2005;11:1867–71.

10. Wu MS, Chen CJ, Lin MT, et al. Genetic polymorphisms of cytochrome p450 2E1, glutathione *S*-transferase M1 and T1, and susceptibility to gastric carcinoma in Taiwan. Int J Colorectal Dis 2002;17:338–43.

11. La Torre G, Boccia S, Ricciardi G. Glutathione S-transferase M1 status and gastric cancer risk: a meta-analysis. Cancer Lett 2005;217:53–60.

12. Hein DW, Doll MA, Fretland AJ, et al. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation. Cancer Epidemiol Biomarkers Prev 2000;9:29–42.

13. Oda Y, Kobayashi M, Ooi A, Muroishi Y, Nakanishi I. Genotypes of glutathione *S*-transferase M1 and *N*-acetyltransferase 2 in Japanese patients with gastric cancer. Gastric Cancer 1999;2:158–64.

14. Ladero JM, Agundez JA, Olivera M, et al. *N*-acetyltransferase 2 single-nucleotide polymorphisms and risk of gastric carcinoma. Eur J Clin Pharmacol 2002;58:115–8.

15. Boissy RJ, Watson MA, Umbach DM, et al. A pilot study investigating the role of NAT1 and NAT2 polymorphisms in gastric adenocarcinoma. Int J Cancer 2000;87:507–11.