

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

Antonio Agudo,¹ Núria Sala,² Guillem Pera,¹ Gabriel Capellá,³ Antonio Berenguer,¹ Nadia García,^{2,3} Domenico Palli,⁴ Heiner Boeing,⁵ Giuseppe Del Giudice,⁶ Calogero Saieva,⁴ Fatima Carneiro,⁷ Franco Berrino,⁸ Carlotta Sacerdote,⁹ Rosario Tumino,¹⁰ Salvatore Panico,¹¹ Göran Berglund,¹² Henrik Simán,¹² Roger Stenling,¹³ Göran Hallmans,¹⁴ Carmen Martínez,¹⁵ Pilar Amiano,¹⁶ Aurelio Barricarte,¹⁷ Carmen Navarro,¹⁸ Jose R. Quirós,¹⁹ Naomi Allen,²⁰ Tim Key,²⁰ Sheila Bingham,²¹ Kay-Tee Khaw,²¹ Jakob Linseisen,²² Gabriele Nagel,²² Kim Overvad,²³ Anne Tjønneland,²⁴ Anja Olsen,²⁴ H. Bas Bueno-de-Mesquita,²⁵ Hendrick C. Boshuizen,²⁶ Petra H. Peeters,²⁷ Mattijs E. Numans,²⁷ Françoise Clavel-Chapelon,²⁸ Marie-Christine Boutron-Ruault,²⁸ Antonia Trichopoulou,²⁹ Eiliv Lund,³⁰ Hendrik Bläker,³¹ Mazda Jenab,³² Pietro Ferrari,³² Teresa Norat,³² Elio Riboli,³³ and Carlos A. González¹

¹Unit of Epidemiology, Catalan Institute of Oncology; ²Medical and Molecular Genetics Center, Oncology Research Institute; ³Laboratory of Translational Research, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge, Hospitalet de Llobregat, Spain; ⁴Epidemiology Unit, Centre for Cancer Research and Prevention, Florence, Italy; ⁵German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany; ⁶Immunobiological Research Institute of Siena, Chiron SPA, Siena, Italy; ⁷Institute of Molecular Pathology and Immunology, University of Porto and Medical Faculty, Porto, Portugal; ⁸Department of Epidemiology, National Cancer Institute, Milan, Italy; ⁹Unit of Cancer Epidemiology, University of Turin and CPO-Piemonte, Turin, Italy; ¹⁰Cancer Registry, Azienda Ospedaliera Civile - M.P. Arezzo, Ragusa, Italy; ¹¹Department of Clinical and Experimental Medicine, Federico II University, Medical School, Naples, Italy; ¹²Malmö Diet and Cancer Study, Lund University, Malmö, Sweden; ¹³Department of Medical Biosciences, Pathology, University of Umea, Sweden; ¹⁴Department of Public Health and Clinical Medicine, Nutritional Research, University of Umea, Sweden; ¹⁵Andalusian School of Public Health, Granada, Spain; ¹⁶Fundación Vasca de Innovación e Investigación Sanitarias, Sondika, Bizkaia, Spain; ¹⁷Public Health Institute, Navarra, Spain; ¹⁸Consejería de Sanidad y Consumo, Murcia, Spain; ¹⁹Dirección General de Salud Pública, Consejería de Salud y Servicios Sanitarios, Asturias, Spain; ²⁰Cancer Research UK Epidemiology Unit, University of Oxford, United Kingdom; ²¹Medical Research Council Dunn Human Nutrition Unit, Cambridge, United Kingdom; ²²Division of Clinical Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg, Germany; ²³Department of Clinical Epidemiology, Aalborg Hospital and Aarhus University Hospital, and Department of Epidemiology and Social Medicine, University of Aarhus, Denmark; ²⁴Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; ²⁵Centre for Nutrition and Health and ²⁶Centre for Information Technology and Methodology, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; ²⁷Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands; ²⁸Institut National de la Santé et de la Recherche Médicale, Institut Gustave Roussy, Villejuif, France; ²⁹Department of Hygiene and Epidemiology, Medical School, University of Athens, Greece; ³⁰Institute of Community Medicine, University of Tromsø, Norway; ³¹Division of General Pathology, University of Heidelberg, Heidelberg, Germany; ³²Unit of Nutrition and Cancer, IARC, Lyon, France; and ³³Cancer Epidemiology and Prevention, Department of Epidemiology and Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom

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 P450 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

Cancer Epidemiol Biomarkers Prev 2006;15(5):1043–5

Received 1/30/2006; accepted 3/2/2006.

Grant support: The EURGAST project received financial support from the FP5 of the European Commission grant QLGI-CT-2001-01049. The EPIC study was funded by Europe Against Cancer Programme of the European Commission, SANCO; Ligue Contre le Cancer, France; Société 3M, France; Mutuelle Générale de l'Éducation Nationale; Institut National de la Santé et de la Recherche Médicale; German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund of the Spanish Ministry of Health grants RCESP-C03/09 and RTICCC 03/10; AGAUR, Generalitat de Catalunya grant 2002-PIR-00333; the participating regional governments and institutions of Spain; Cancer Research UK; Medical Research Council, UK; the Stroke Association, United Kingdom; British Heart Foundation; Department of Health, United Kingdom; Food Standards Agency, United Kingdom; the Wellcome Trust, United Kingdom; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch ZON, Zorg Onderzoek Nederland; World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden; and Norwegian Cancer Society.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Antonio Agudo, Unit of Epidemiology, Catalan Institute of Oncology, Av. Gran Via s/n km 2.7, 08907 L'Hospitalet de Llobregat, Spain. Phone: 34-932607401; Fax: 34-932607787. E-mail: a.agudo@ico.scs.es

Copyright © 2006 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-06-0073

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)
<i>CYP2E1</i>	-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)
<i>GSTM1</i>	del{GSTM1}	Present	120 (49.6)	434 (46.6)	1 (reference)
		Null	122 (50.4)	498 (53.4)	0.88 (0.67-1.17)
<i>NAT1</i>	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)
<i>NAT2</i>	Ex2+487C>T (<i>L161L</i> , *5A) rs1799929	AA/CA	86 (36.6)	338 (36.4)	1.01 (0.75-1.37)
		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)
	Ex2-580G>A (<i>R197Q</i> , *6A) rs1799930	TT/CT	162 (67.5)	636 (68.8)	0.93 (0.69-1.26)
		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)
	Ex2-313G>A (<i>G286E</i> , *7A/B) rs1799931	AA/GA	116 (48.3)	479 (51.9)	0.87 (0.65-1.16)
		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.

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.

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, *RsaI* 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

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.

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)		$P_{\text{interaction}}^*$
			Cases/controls [†]	OR [‡] (95% CI)	Cases/controls [†]	OR [‡] (95% CI)	
<i>CYP2E1</i>	-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
<i>GSTM1</i>	del[GSTM1]	Null vs present	38/223	0.79 (0.48-1.28)	84/275	1.01 (0.70-1.46)	0.45
<i>NAT1</i>	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
<i>NAT2</i>	Ex2+487C>T (<i>L161L</i> , *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 (<i>R197Q</i> , *6A)	AA/GA vs GG	44/221	0.98 (0.60-1.59)	72/258	0.85 (0.59-1.24)	0.61
	Ex2-313G>A (<i>G286E</i> , *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).

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.

Acknowledgments

We thank the members of the pathologist panel for their valuable work: Dr. Johan Offerhaus (Amsterdam, The Netherlands), Dr. Vicki Save (Cambridge, United Kingdom), Dr. Julio Torrado (San Sebastian, Spain), Dr. Gabriella Nesi (Firenze, Italy), Dr. U. Mahlke (Potsdam, Germany), Dr. Claus Fenger (Denmark), and Dr. Dimitrios Roukos (Ioannina, Greece); Catia Moutinho (Porto, Portugal) for her technical work in the preparation of pathologic material; and Francisco Rico and Fátima Marín for their technical assistance in the genotyping analysis.

References

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

- Gonzalez CA, Sala N, Capella G. Genetic susceptibility and gastric cancer risk. *Int J Cancer* 2002;100:249-60.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Cai L, Zheng ZL, Zhang ZF. Cytochrome p450 2E1 polymorphisms and the risk of gastric cardia cancer. *World J Gastroenterol* 2005;11:1867-71.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association
for Cancer Research

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

Antonio Agudo, Núria Sala, Guillem Pera, et al.

Cancer Epidemiol Biomarkers Prev 2006;15:1043-1045.

Updated version Access the most recent version of this article at:
<http://cebp.aacrjournals.org/content/15/5/1043>

Cited articles This article cites 13 articles, 3 of which you can access for free at:
<http://cebp.aacrjournals.org/content/15/5/1043.full#ref-list-1>

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at:
<http://cebp.aacrjournals.org/content/15/5/1043.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/15/5/1043>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.