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Glutathione S-transferase M1 and T1 genetic polymorphisms are not related to the risk of hepatocellular carcinoma: A study in the Spanish population

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ABSTRACT

Glutathione S-transferases constitute a superfamily of enzymes that catalyse the inactivating conjugation of endogenous and environmental substrates involved in the pathogenesis of hepatocellular carcinoma (HCC) and glutathione. Genes encoding either glutathione S-transferase Mu-1 or Theta-1 (*GSTM1* and *GSTT1*, respectively) isoforms are polymorphic. Homozygotes for the mutated inactive alleles of each gene are devoid of any specific enzymatic activity (null genotypes). Our aim was to investigate whether individuals with null GST genotypes have a higher risk of developing HCC. A total of 184 Caucasian Spanish patients with a diagnosis of HCC and 329 healthy controls of the same ethnic origin were included. Polymorphisms in *GSTM1* and *GSTT1* genes were identified through multiplex polymerase chain reactions, and the dihydrofolate reductase (*DHFR*) gene was used as internal control. No differences were found between the frequencies of *GSTM1* (47.8% versus 45.3%) and *GSTT1* (28.8% versus 23.1%) null genotypes in cases and controls, respectively, nor in the proportion of carriers of two, one or no active genotypes. Gender, age at diagnosis, tobacco use, chronic infection with hepatitis B or C virus and alcohol abuse did not influence these results. In conclusion, polymorphisms in *GSTM1* and *GSTT1* genes are not related to the incidence of HCC in a high-risk Spanish population.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, with an estimated incidence rate of more than 500,000 new cases per year [1]; there are considerable geographical differences in frequency and aetiological factors of HCC [2,3]. The incidence of HCC is increasing in the USA [4], but the picture is more mixed in Europe: the incidence nearly tripled in France between 1980 and 2000 [5] and a lesser increase, limited to men aged over 75 years, has been observed in the Netherlands [6]. Global data in Spain show a slight decline between 1970 and 1996 [7], although there were no changes in HCC incidence in the autonomous community of Catalonia in the period 1980–1997 [8]. Some studies suggest that this neoplasm is the leading cause of death among cirrhotic patients in Europe [9], where the most important risk factor for HCC is liver cirrhosis [10,11], mainly in cases due to chronic infection with hepatitis C virus (HCV) [12,13],

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alcohol abuse [2,3,13] or haemochromatosis [14]. Environmental or acquired factors, other than alcohol abuse, have been recognised as risk factors for HCC in cirrhotic patients, for example, tobacco use [15], diabetes mellitus [16] and obesity [17]. Many of these factors may act synergistically to increase the risk of HCC [15,18].

Interestingly, only a minority of patients at risk develops HCC. As in many other diseases of multifactorial origin, the combination of genetic and environmental factors may be involved in the pathogenesis of HCC. Genetic polymorphisms affecting the metabolic pathways of activation or neutralisation of carcinogens have been studied in many cancers with conflicting, but occasionally significant, results [19]. Today, the influence of genes encoding for drug and xenobiotic-metabolising enzymes is recognised, and these are considered low-penetrance genes in cancer risk. Glutathione S-transferases (GST) are a superfamily of detoxifying enzymes involved in the neutralisation of endogenous by-products of oxidative stress and exogenous chemicals of proven carcinogenicity [20–24]. It has been suggested that GST enzymes may be involved in susceptibility to cancer [23].

Of the various GST isoenzyme classes identified so far, two are coded by polymorphic genes: *GSTM1* and *GSTT1*. The former codes for the GST-μ isoform and is located at chromosome 1p13.3, whereas the *GSTT1* gene locus in 22q11.2 codes for the GST-θ isoform. In both genes, a partial deletion of the coding sequence causes the total absence of enzymatic function (null alleles). Both polymorphic traits are inherited in an independent manner. Homozygotes for null alleles of either gene are devoid of specific enzymatic activity in all their organs and tissues [23,24]. There is no evidence of whether heterozygosity in either *GSTM1* or *GSTT1* affects gene function [25].

Carriers of the *GSTM1* and/or *GSTT1* null genotypes may have a reduced ability to inactivate electrophilic compounds and organic hydroperoxides in the liver [26,27] and, more specifically, polycyclic aromatic hydrocarbons present in tobacco smoke [21] and aflatoxin B [28]. This provides a biological basis for a putative role of GST polymorphisms in the risk of developing cancers related to these substances. The role of *GSTM1* and *GSTT1* polymorphisms in colorectal, lung, bladder and other cancers has been studied with conflicting results [19,25,29]. Our aim was to establish the frequency of *GSTM1* and *GSTT1* null genotypes in Spanish patients with HCC, in order to clarify whether any relationship exists between these polymorphisms and the risk of developing this tumour.

2. Patients and methods

2.1. Study population

We studied a cohort of 184 unrelated Caucasian patients of Spanish ancestry and nationality, diagnosed with HCC at the Department of Gastroenterology of the Hospital Clínico San Carlos, Madrid, Spain, between January 1994 and September 2004. One hundred and fifty patients were male (mean age ± SD at diagnosis 65.3 ± 7.0 years) and 34 were female (70.4 ± 9.0 years).

The diagnosis of HCC was suspected from the identification of a mass in the liver and was confirmed by histopathological studies (aspirative cytology and/or surgical specimen) in 79 cases, a blood level of alpha-foetoprotein > 399 ng/dl (70 cases) or both (35 cases). Data regarding known previous liver disease, alcohol and tobacco consumption, serum tests for hepatitis B and C virus (not available in 9 and 6 cases, respectively) and other diseases were collected. Heavy drinkers were defined as individuals drinking more than 50 g of alcohol per day.

The control group comprised 329 unrelated healthy Caucasian Spanish individuals from the same geographical area (198 males, mean age 42.1 ± 11.5 years and 131 females, mean age 44.6 ± 14.5 years). A medical history and laboratory tests were obtained from each individual to exclude pre-existing conditions.

The study protocol was approved by the local ethics committee and all subjects gave informed consent before inclusion in the study.

2.2. Molecular analyses

Genotype analyses were carried out in genomic DNA obtained from blood samples. A multiplex polymerase chain reaction (PCR) assay was used to determine the *GSTM1* and *GSTT1* genotypes. The dihydrofolate reductase (*DHFR*) gene was co-amplified as an internal control, as described elsewhere [30]. A minor modification in the protocol was as follows: multiplex PCR reactions were carried out in a final volume of 12 μl. The primers used were those described by Xiong and colleagues [30]. PCR products of 480, 215 and 280 base pairs revealed the presence of *GSTT1*, *GSTM1* and *DHFR* genes, respectively. When none of these bands were present, the samples were discarded because *DHFR* is an essential gene that should amplify in all samples.

2.3. Statistical analyses

Differences between patients and controls in the distribution of *GSTM1* and *GSTT1* genotypes, which were considered dichotomical variables, were analysed by the Mantel–Haenszel χ^2 test with Bonferroni's correction, or by the Fisher exact test, when appropriate. The odds ratios with their corresponding 95% confidence intervals (CIs) were calculated simultaneously. Continuous variables were compared using the Student's *t*-test or the Mann–Whitney *U* test, when appropriate. All tests were two-sided. The calculations were made with the SPSS 11.5 statistical software package for Windows and the Epi-Info 6 software package of the Centers for Disease Control in Atlanta. The null hypothesis was rejected when $P < 0.05$.

3. Results

The distribution of GST genotypes in both groups is shown in Table 1. No significant differences were observed between cases and controls in the proportion of *GSTM1* (47.8% versus 45.3%) and *GSTT1* (28.8% versus 23.1%) null genotypes, nor were differences found in the number of active genotypes.

Table 1 – GSTM1 and GSTT1 genotype distribution and number of glutathione S-transferase (GST) active genotypes in patients with hepatocellular carcinoma and in normal controls

	GSTM1 genotype (%)		*GSTT1* genotype (%)		Number of active genotypes (%)		
	Null	Active	Null	Active	Two	One	None
Cases	88 (47.8)	96 (52.2)	53 (28.8)	131 (71.2)	63 (34.2)	101 (54.9)	20 (10.9)
Controls	149 (45.3)	180 (54.7)	76 (23.1)	253 (76.9)	128 (38.9)	177 (49.3)	24 (7.3)
Statistics	OR 1.11		OR = 1.35		OR 0.82[a]	OR 1.07[a]	OR = 1.46[a]
	95% CI 0.76–1.62		95% CI = 0.88–2.07		95% CI 0.58–1.21	95% CI 0.73–1.56	95% CI 0.74–2.87
	MH 0.31 $P = 0.58$		MH 2.04 $P = 0.15$		MH 1.10 $P = 0.29$	MH 0.13 $P = 0.72$	MH 1.41 $P = 0.24$

OR, odds ratio; 95% CI, 95% confidence interval; MH, Mantel–Haenszel χ^2 test.
a After Bonferroni's correction for two degrees of freedom.

Table 2 – Distribution of GSTs genotypes by gender and aetiological categories[a]

Group (no. of cases)	*GSTT1*		*GSTM1*		Double null (%)
	Active (%)	Null (%)	Active (%)	Null (%)	
Controls (329)	253 (76.9)	76 (23.1)	180 (54.7)	149 (45.3)	24 (7.3)
Cases (184)	131 (71.2)	53 (28.8)	96 (52.2)	88 (47.8)	20 (10.9)
Male gender (150)	109 (72.2)	41 (27.3)	77 (51.3)	73 (48.7)	18 (12)
HCV + (119)	85 (71.4)	34 (28.6)	68 (57.1)	51 (42.9)	12 (10.1)
HbsAg + (12)	9.0 (75)	3 (25.0)	8.0 (66.7)	4.0 (33.3)	0.0 (0)
Alcohol > 50 g (81)	62 (76.5)	19 (23.5)	41 (50.6)	40 (49.4)	9 (11.1)
Alcohol > 50 g virus-free (30)	22 (73.3)	8 (26.7)	14 (46.7)	16 (53.5)	5 (16.7)[b]
Alcohol > 50 g and HCV + (46)	37 (80.4)	9 (19.6)	26 (57.8)	20 (43.5)	3 (6.5)

a No significant differences exist between controls, the whole group of patients and the different subgroups.
b $P = 0.081$ (Fisher exact test). HCV, hepatitis C virus; HbsAg, hepatitis B surface antigen; GST, glutathione S-transferase.

Table 2 presents the distribution of individual GST genotypes and the number of active genotypes in several subgroups of patients classified by gender, heavy alcohol consumption and/or chronic HCV infection. Again, no significant differences were observed, although the proportion of heavy drinkers free of chronic viral infection with two null genotypes was more than twice the frequency present in the control group ($P = 0.081$, Fisher exact test).

Age at diagnosis, tobacco use, hepatitis B surface antigen (HbsAg) status and the absence of any known cause of chronic liver disease did not influence the distribution of the studied genotypes (data not shown).

4. Discussion

An increasing body of evidence points to a combination of environmental factors and genetic susceptibility as a major determinant for the development of several forms of cancer. In this context, impairment of detoxifying enzymes seems to be a relevant genetic factor. It can be hypothesised that individuals with impaired metabolism of dietary or environmental carcinogenic substances are at greater risk of developing cancer if they are exposed to such carcinogenic substances. In this regard, it should be pointed out that dietary and environmental factors related to HCC vary greatly between different human populations. In Oriental subjects, where aflatoxin B1 is a common cause of HCC, a preliminary study of *GSTM1* polymorphism including only 30 HCC patients showed no differences in the frequency of null genotypes between patients and controls [31]; only 1 year later, however, study of the same group suggested that both the *GSTM1* and *GSTT1* null genotypes might increase the risk of HCC in chronic HbsAg carriers if they are simultaneously exposed to aflatoxin B [32]. A third study including 79 HCC patients detected a higher risk associated with the *GSTT1* null genotype in combination with aflatoxin exposure, but the influence of *GSTM1* null genotype was not significant [33]. Subsequently, the same group reported that the influence of the *GSTT1* null genotype was higher in carriers of the –399 Gln allele of the *XRCC1* gene, which is involved in DNA repair. The mutated *XRCC1* allele is associated with lower efficiency of DNA repair and with elevated aflatoxin B1-DNA adduct levels; surprisingly, the *GSTM1* null genotype was associated with a decreased risk of early-onset HCC [34]; all the patients included in that study (577) were also chronic hepatitis B virus (HBV) carriers. All these studies were performed in Taiwan and suggest that GST polymorphisms, mainly *GSTT1* null genotype, may be related to the risk of HCC in chronic HBV carriers exposed to aflatoxin, especially if they are associated to other predisposing genetic traits. Studies carried out in Sudan revealed a positive association between consumption of peanut butter, which supposedly contains significant amounts of aflatoxin, and the risk of HCC only in *GSTM1* null genotype patients [35], but not in those carrying the *GSTT1* null genotype [36].

Other studies also performed in Far Eastern countries, which did not evaluate aflatoxin exposure, failed to show any relationship [37] or only a minimal influence of the *GSTM1* null genotype on the risk associated with a combination of smoking, drinking and low plasma carotenoid levels [38].

The aetiology of HCC in Western countries is completely different from that found in Eastern Asia and sub-Saharan Africa. Aflatoxin exposure is negligible and the role of chronic HBV infection is relatively small. In contrast, the majority of patients in Western countries suffer from cirrhosis due to HCV chronic infection and/or alcohol abuse. Both these factors may generate highly reactive compounds that induce oxidative damage in the liver, as demonstrated by increased levels of malonyl dialdehyde and 4-hydroxy-nonenal found in chronic hepatitis C [39,40] and in alcoholic liver disease [41]. The detoxifying action of glutathione S-transferases may neutralise these toxic by-products [41,42]. Thus, a decreased detoxifying rate linked to the GST null genotypes may be related to the risk of developing HCC as the most severe form of liver damage induced by chronic HCV infection or alcohol abuse.

In a recent report by Gelatti and colleagues [43] on a study performed in Italy, no relationship was found between *GSTM1* and *GSTT1* genotypes and the risk of HCC. The specific aim of that study was to establish whether the polymorphisms studied influence susceptibility to HCC in relation to smoking, though the authors did not analyse in detail the role of alcohol abuse in subjects lacking other HCC risk factors, as we did in the present study.

We found no relationship between *GSTM1* and *GSTT1* genetic polymorphisms and the risk of HCC in the population studied. However, the increased proportion of double null genotypes found in patients in whom alcohol abuse was the only identified risk factor for developing the disease is in agreement with our previous finding of an excess of double null genotypes among alcoholic cirrhotics who had not developed hepatocellular carcinoma [44], although this difference does not reach statistical significance due to the small size of the group.

From our observations, we conclude that polymorphisms of both glutathione S-transferase μ1 and θ1 genes are not related to the risk of hepatocellular carcinoma in a high-risk Spanish population, and in particular among patients who developed HCC after chronic HCV infection. In contrast, our findings suggest that in certain individuals who developed HCC as a consequence of alcohol abuse only, null genotypes may influence the risk of developing HCC, and this concurs with the hypothesis for a combined effect of genetic and environmental factors. Once we have shown that GST null genotypes do not influence the risk of developing HCC in the majority of subjects, further studies will be necessary to establish the role of null genotypes in a larger group of HCC patients with no risk factors except alcohol abuse.

Conflict of interest statement

None declared.

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REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al. Estimating the world cancer burden: GLOBOCAN 2000. *Int J Cancer* 2001;**94**:153–6.
2. Bosch FX, Ribes J, Borràs J. Epidemiology of primary liver cancer. *Semin Liver Dis* 1999;**19**:271–85.
3. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;**362**:1907–17.
4. Howe HL, Wingo PA, Thun MJ, et al. Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. *J Natl Cancer Inst* 2001;**93**:824–42.
5. Bouvier AM, Remontet L, Jougla E, et al. Incidence of gastrointestinal cancers in France. *Gastroenterol Clin Biol* 2004;**28**:877–81.
6. Verhoef C, Visser O, de Man RA, et al. Hepatocellular carcinoma in the Netherlands. Incidence, treatment and survival patterns. *Eur J Cancer* 2004;**40**:1530–8.
7. La Vecchia C, Lucchini F, Franceschi S, et al. Trends in mortality from primary liver cancer in Europe. *Eur J Cancer* 2000;**36**:909–15.
8. Ribes J, Clèries R, Borràs J, et al. Time trends and mortality for chronic liver disease and liver cancer in the interval 1980–1997 in Catalonia, Spain. *Eur J Gastroenterol Hepatol* 2004;**16**:865–72.
9. Fattovich G, Glustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;**19**:271–85.
10. Simonetti RG, Cammà C, Fiorello F, et al. Hepatocellular carcinoma. A worldwide problem and the major risk factors. *Dig Dis Sci* 1991;**36**:862–72.
11. Sangro B, Herráiz M, Martínez-González MA, et al. Prognosis of hepatocellular carcinoma in relation to treatment: a multivariate analysis of 178 patients from a single European institution erratum in Surgery 1998, 124, 1087. *Surgery* 1998;**124**:575–83.
12. Bruix J, Barrera JM, Calvet X, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989;**2**:1004–6.
13. Donato F, Tagger A, Chiesa R, et al. Hepatitis B and C virus infection, alcohol drinking and hepatocellular carcinoma: a case-control study in Italy. *Hepatology* 1997;**26**:579–84.
14. Elmberg M, Hultcrantz R, Ekbom A, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology* 2003;**125**:1733–41.
15. Marrero JA, Fontana RJ, Fu S, et al. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol* 2005;**42**:218–24.
16. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004;**126**:460–8.
17. Calle EE, Rodríguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *N Engl J Med* 2003;**348**:1625–38.

18. Hassan MM, Hwang LY, Hatten CJ, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002;**36**:1206–13.
19. d'Errico A, Taioli E, Chen P, et al. Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers* 1996;**1**:149–73.
20. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST* and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995;**30**:445–600.
21. Hirvonen A. Genetic factors in individual responses to environmental exposures. *J Occup Environ Med* 1995;**37**:37–43.
22. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000;**61**:154–66.
23. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 1997;**6**:733–43.
24. Pemble S, Schroeder KR, Spencer SR, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994;**300**:271–6.
25. Cotton SC, Sharp L, Little J, et al. Glutathione S-transferase polymorphisms and colorectal cancer: a HuGE review. *Am J Epidemiol* 2000;**151**:7–32.
26. Rowe JD, Nieves E, Listowsky I. Subunit diversity and tissue distribution of human glutathione S-transferases: interpretation based on electrospray ionization-MS and peptide sequence-specific antisera. *Biochem J* 1997;**325**:481–6.
27. Haufroid V, Jakubowski M, Janasik B, et al. Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological exposure to styrene. *Pharmacogenetics* 2002;**12**:691–702.
28. Eaton DL, Bammler TK, Kelly EJ. Interindividual differences in response to chemoprotection against aflatoxin-induced hepatocarcinogenesis: implications for human biotransformation enzyme polymorphisms. *Adv Exp Med Biol* 2001;**500**:559–76.
29. Eaton DL, Bammler TK. Glutathione S-transferases. In: Levy KE, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 175–89.
30. Xiong P, Bondy ML, Li D, et al. Sensitivity to benzo(a)pyrene diol-epoxide associated with risk of breast cancer in young women and modulation by glutathione s-transferase polymorphisms: a case-control study. *Cancer Res* 2001;**61**:8465–9.
31. Yu MW, Gladek-Tarborough A, Chiampraset S, et al. Cytochrome p450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. *Gastroenterology* 1995;**109**:1266–73.
32. Chen CJ, Yu MW, Liaw YF, et al. Chronic hepatitis B carriers with null genotypes of glutathione S-transferase M1 and T1 polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma. *Am J Hum Genet* 1966;**59**:128–34.
33. Sun CA, Wang LY, Chen CJ, et al. Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan. *Carcinogenesis* 2001;**22**:1289–94.
34. Yu MW, Yang SY, Pan IJ, et al. Polymorphisms in XRCC1 and glutathione S-transferase genes and hepatitis B-related hepatocellular carcinoma. *J Natl Cancer Inst* 2003;**95**:1485–8.
35. Omer RE, Verhoef L, Van't Veer P, et al. Peanut butter intake, GSTM1 genotype and hepatocellular carcinoma: a case-control study in Sudan. *Cancer Causes Control* 2001;**12**:23–32.
36. Tiemersma EW, Omer RE, Bunschoten A, et al. Role of genetic polymorphism of glutathione-S-transferase T1 and microsomal epoxide hydrolase in aflatoxin-associated hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:785–91.
37. Munaka M, Koshsi K, Kawamoto T, et al. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2003;**129**:355–60.
38. Yu MW, Chiu YH, Chiang YC, et al. Plasma carotenoids, glutathione S-transferase M1 and T1 genetic polymorphisms, and risk of hepatocellular carcinoma: independent and interactive effects. *Am J Epidemiol* 1999;**149**:62162–9.
39. De Maria N, Colantoni A, Fagiuoli S, et al. Association between reactive oxygen species and disease activity in chronic hepatitis C. *Free Radic Biol Med* 1996;**21**:291–5.
40. Loguercio C, Federico A. Oxidative stress in viral and alcoholic hepatitis. *Free Radic Biol Med* 2003;**34**:1–10.
41. Day CP. Who gets alcoholic liver disease: nature or nurture? *JR Coll Physicians Lond* 2000;**34**:557–62.
42. Hartley DP, Ruth JA, Petersen DR. The hepatocellular metabolism of 4-hydroxynonenal by alcohol dehydrogenase, aldehyde dehydrogenase, and glutathione S-transferase. *Arch Biochem Biophys* 1995;**316**:197–205.
43. Gelatti U, Covolo L, Talamini R, et al. N-acetyltransferase-2, glutathione S-transferase M1 and T1 genetic polymorphisms, cigarette smoking and hepatocellular carcinoma: a case-control study. *Int J Cancer* 2005;**115**:301–6.
44. Ladero JM, Martínez C, García-Martín E, et al. Polymorphisms of the glutathione S-transferases Mu-1 (GSTM1) and theta-1 (GSTT1) and the risk of advanced alcoholic disease. *Scand J Gastroenterol* 2005;**40**:348–53.