

Genetic Polymorphisms of Cytochrome P4501A1 (*CYP1A1*) and Glutathione *S*-Transferase P1 (*GSTP1*) and Risk of Hepatocellular Carcinoma Among Chronic Hepatitis C Patients in Egypt

Ekbal M. Abo-Hashem¹ · Wafaa M. El-Emshaty¹ ·
Raghda El Sayed Farag² · Sahar Zakaria² ·
Mohammed Abd El-Aziz² · Azza Ghonaim³

Received: 16 September 2015 / Accepted: 27 May 2016 / Published online: 7 June 2016
© Springer Science+Business Media New York 2016

Abstract Cytochrome P450 1A1 (*CYP1A1*) and Glutathione *S*-transferase P1 (*GSTP1*) genes are involved in the metabolism of many carcinogens. Polymorphisms in these genes with altered enzyme activity have been reported. The present study evaluated the synergistic effect between *CYP1A1* and *GSTP1* gene polymorphisms and smoking on development of HCV-related liver disease and hepatocellular carcinoma (HCC). The patients group comprised 40 patients with HCC and 40 patients with liver cirrhosis. The control group comprised 40 healthy subjects having no history of malignancy. The genetic polymorphisms were studied using polymerase chain reaction restriction fragment length polymorphism (PCR RFLP) technique on blood samples. The number of current or former smoker among HCC and cirrhotic patients as well as the median Pack/year of cigarette smoked were significantly higher in HCC and liver cirrhotic patients than in control group. Subjects with *CYP1A1* gene variants (m1 and m3) had no significant risk to develop cirrhosis or HCC compared to control group. Individuals carrying the *Ile/Val* genotype of *GSTP1* had a significant increased risk of HCC (OR of 2.2, 95 % CI 1.143–4.261) and had larger tumor size. No significant risk was observed on combining both genes variants or on combining smoking with variants of both genes. In conclusion, the *GSTP1 Ile/Val* genotype and *Val* allele are associated with an increased risk of HCC. *CYP1A1* and *GSTP1* genes variants interaction did not increase the risk of HCC.

Keywords *GSTP1* · *CYP1A1* · HCC · HCV · Smoking

✉ Wafaa M. El-Emshaty
dr.wafaelemshaty@yahoo.com

¹ Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt

² Department of Tropical Medicine, Mansoura University, Mansoura, Egypt

³ Department of Clinical Pathology, Mansoura New General Hospital, Mansoura, Egypt

Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent malignant tumor worldwide and ranks the third as a cancer killer, causing about half million deaths annually (Jemal et al. 2011). In Egypt, an overall increase in the relative frequency of all liver-related cancers (>95 % as HCC), from approximately 4 % in 1993 to 7.3 % in 2003 has been reported (El-Zayadi et al. 2005).

Human hepatocarcinogenesis is a multistage process with multiple risk factors. Chronic hepatitis B and C play a major role in HCC etiology. Other risk factors including alcohol consumption, male gender, obesity, aflatoxin, or other chemicals may be involved (El-Serag and Rudolph 2007; Gross-Steinmeyer and Eaton 2012).

Tobacco smoke contains many carcinogens. Most of which require metabolic activation mainly through aryl hydrocarbon hydroxylase activity encoded by phase I drug-metabolizing enzymes, such as cytochrome P450s (*CYPs*). Cytochrome P-450 1A1 (*CYP1A1*) (cytochrome P450, family1, subfamily A, polypeptide1) is the most active among the *CYPs* enzyme in the metabolism of pro-carcinogenic tobacco components (Nerurkar et al. 2000) into hydrophilic, reactive, electrophilic intermediate forms capable of DNA binding that may initiate the carcinogenic process (National Research Council 1983).

Four genotypes of *CYP1A1*, resulting from various point mutations in the *CYP1A1* gene, have been characterized and designated *m1*, *m2*, *m3*, and *m4*. Both *m1* (rs4646903) variant, a T → C substitution at nucleotide 3801 and *m3* (rs4986883) variant, nucleotide 3205 T → C, create an *MspI* restriction site in the 3'-non-coding region (Cascorbi et al. 1996).

Tobacco metabolic intermediates are further detoxified by phase II enzymes such as glutathione *S*-transferase (*GST*) (Smith et al. 1995) that protect normal cells against damage induced by hepatitis virus or aflatoxin. The family is composed of eight isoforms (Kato et al. 2008). *GSTP1* plays a central role in the inactivation of toxins and carcinogens (Hengstler et al. 1998). A single nucleotide polymorphism in the *GSTP1* gene causes substitution of isoleucine to valine at amino acid codon 105 (*Ile105Val*) is reported (Allan et al. 2001).

Polymorphisms in genes encoding *CYP1A1* or *GSTP1* enzymes, possibly by altering their expression and function, may increase or decrease carcinogen activation or detoxification and modulate DNA repair, and are implicated in various cancers (Yu et al. 1999; Kweekel et al. 2008). However, results from other studies have revealed inconsistent findings and remain conflicting rather than conclusive (Silvestri et al. 2003; Li et al. 2009; Huang et al. 2013).

These inconsistent findings justify the need for additional studies to further evaluate the role of *CYP1A1* and *GSTP1* polymorphism—and their possible interaction as risk factors for HCC development among Egyptians and its relation to smoking. Hence, this case–control study was planned.

Materials and Methods

Subjects and Specimen Collection

This study was conducted on 40 patients with HCC and 40 patients with liver cirrhosis. Patients were recruited from the Department of Tropical medicine, Mansoura University Hospital between March 2013 and August 2014. The diagnosis of HCC was confirmed by α -fetoprotein elevation (>400 ng/ml) combined with one positive imaging study [Magnetic resonance imaging (MRI) or computerized tomography (CT)] or two positive imaging studies with normal α -fetoprotein level or a pathological examination when needed (Ryder 2003).

Diagnosis of chronic hepatitis and cirrhosis was based on biochemical markers, liver serology, imaging studies, and histopathological scoring assessment. Forty control subjects were included in the study. The selection criteria for control subjects included the absence of current or previous history of any kind of malignancy and age matching to HCC cases. Patients and control subjects gave their written informed consent to participate in the study. The Ethical Research Committee of Mansoura University approved the study protocol.

For all cases and controls, full clinical history and thorough clinical examination were carried out. Clinical history included history of smoking, alcohol consumption, and liver disease symptoms.

Subjects who smoked more than one cigarette per day for more than 1 year were classified as smokers. Others were defined as non-smokers. The average number of cigarettes smoked per day and the total number of years of smoking were used to calculate cumulative smoking dose as 'pack-years' (PY) [(cigarettes per day/20) \times years smoked]. Subjects were also categorized as light (<30 PY) or heavy (≥ 30 PY) smokers, based on the mean tobacco consumption in the control group.

Venous blood samples were collected on EDTA tube (4 ml) and plain tube (4 ml) from all participants. Sera were separated from the plain tubes and distributed into aliquots that were used for the detection of serum HBV markers, antibodies to hepatitis C virus [DiaSorin ELISA (Italy) commercial kits], liver biochemical profile, and alpha-fetoprotein. Serum albumin, bilirubin, ALT, and AST were tested using automated chemistry analyzer Cobas C111 (Roche-Germany). Alpha-fetoprotein estimation was done by electrochemiluminescence technique by automated analyzer Cobas e (Roche, Germany). EDTA blood samples were used for complete blood count and immediately stored at -80 °C for the extraction of genomic DNA and detection of genetic polymorphism in the *CYP1A1* and *GSTP1* genes using PCR RFLP. For each patient in liver cirrhosis or HCC group, calculation of Child-Pugh score was carried out to assess the prognosis of chronic liver disease. The score employs five clinical measures (serum total bilirubin, serum albumin, prothrombin time, the presence of ascites and hepatic encephalopathy). Each measure is scored 1–3, with 3 indicating most severe derangement (Cholongitas et al. 2005). For all HCC patients, BCLC score was assessed (Fornier et al. 2014).

Genomic DNA Extraction

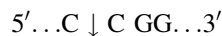
Genomic DNA was extracted from whole blood using Gene JET whole-blood genomic DNA purification Mini Kit (#k0781). The kit utilizes silica-based membrane technology in the form of a convenient spin column.

Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP)

The first mutation analyzed in the *CYP1A1* gene was 6235 T → C transition 1194 bp downstream of axon 7 (m1 mutation). The other mutation analyzed was 5639 T → C transition downstream of axon 7 (m3 mutation). For *GSTP1* gene, the mutation analyzed was 313A → G transition (rs1695, *GSTP1* Ile I05 Val).

Of *CYP1A1* and *GSTP1* genes, an 899-bp and 440-bp DNA fragment was separately amplified using 1 unit Taq polymerase, 10 μmol/l of primers M3F 5'-GGCTGAG CAATCTGACCCTA, and P80, 5'-TAGGAGTCTTGTCTCATGCCT, for m1 and m3 *CYP1A1* or of primers 5'-ACG CAC ATC CTC TTC CCC TC 3' and 5'-TCA TTC TTG GCT GGT TGA TGT CC 3' for *GSTP1*, 0.2 mmol/l deoxynucleotide triphosphate, and 2.4 mmol/l MgCl₂ in a total volume of 30 μl. All primers were obtained from Thermo Fisher Scientific Inc. PCR reactions were carried out with a Perkin-Elmer Applied Biosystems 9600 thermal cycler. PCR conditions for *CYP1A1* were initial denaturation for 3 min at 95 °C followed by 35 cycles of 0.5 min at 94 °C, 1 min at 63 °C, and 1 min at 72 °C. Final extension was at 72 °C for 10 min, while that for *GSTP1* were initial denaturation for 3 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 57 °C, and 30 s at 72 °C. Final extension was at 72 °C for 7 min.

The PCR product was digested with 50 units *MspI* or *BsmAI* (Thermo Scientific Fast digest restriction enzyme), for *CYP1A1* and *GSTP1* genes, respectively, generating smaller fragments in case of the mutation. Thermo Scientific *MspI* enzyme restriction site is



while Thermo Scientific *BsmAI* enzyme restriction site is



The following reaction components were combined at room temperature: nuclease-free water (17 μl), 10× fast digest green buffer (2 μl), amplified PCR product (10 μl), and fast digest enzyme (1 μl) in a total volume of 30 μl. The components were mixed and incubated at 37 °C in a heat block for 15 min. The PCR product and the fragments of restriction enzyme digestion were evaluated on an ethidium bromide-stained 2 % agarose gel.

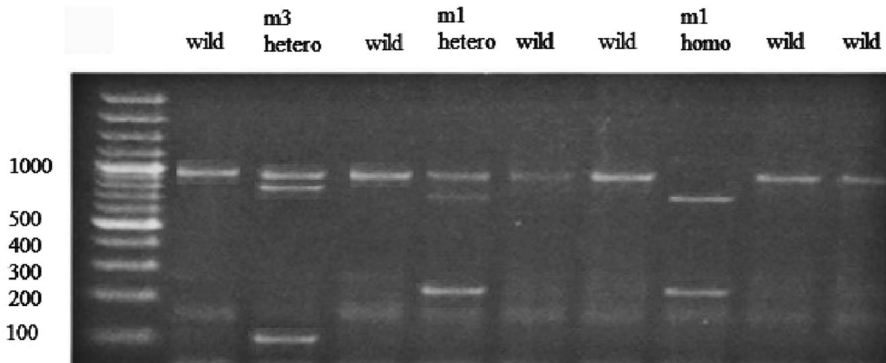


Fig. 1 Agarose gel electrophoresis stained with ethidium bromide. Showing bands of *CYP1A1* gene digested PCR product with *MspI* restriction enzyme using 100 bp ladder marker, showing wild type with fragment length 899 bp; m1 heterozygous (wild/m1 mutant) with fragments 899, 693, and 206 bp; m3 heterozygous (wild/m3 mutant) with fragments 802, 97, 899; and m1 homozygous (m1/m1) with fragments 693, 206 bp

The sizes of PCR products were estimated according to the migration pattern of a 100 and 50 bp DNA ladder for *CYP1A1* and *GSTP1* genes, respectively (Thermo Scientific Gene ruler plus DNA ladder).

Genotypes for *CYP1A1* gene were determined (Fig. 1) as homozygous for the wild-type allele (w/w; 899 bp), heterozygous (w/m1; 899, 693, 206 bp or w/m3; 802, 97, 899), or homozygous for mutant allele (m1/m1 693, 206 bp or m3/m3; 802, 97).

The A to G polymorphism of *GSTP1* introduces a restriction site recognized by the *BsmAI* restriction enzyme results either in retention of the 440 bp product or complete digestion to 212 and 228 bp fragments corresponding to individuals homozygous for the Ile or Val alleles, respectively. The presence of all three fragments corresponded to individuals heterozygous (Ile/Val), at codon 105 (Fig. 2).

Statistical Analysis

The statistical analysis of data was done using statistical package for social science program (SPSS, Inc, Chicago, IL) version 20. The normality of data distribution was tested by Kolmogorov–Smirnov test. Qualitative data were presented as frequency and percentage. Chi-square and Fisher exact tests were used to compare groups. Quantitative data were presented as minimum, maximum, median or mean and standard deviation. For comparison between groups, student *t* test and Mann–Whitney and Kruskal–Wallis tests were used as appropriate. Deviations from Hardy–Weinberg equilibrium expectations (HWE) were determined using logistic regression analysis. Bonferroni’s correction was used for multiple corrections. Odds ratio and 95 % confidence interval were calculated. Gene–gene interaction analysis was conducted, using as a reference group the homozygous wild-type individuals; while for gene–environment interaction analyses, the reference group was wild-type homozygous non-smoker. Multifactorial dimensional reduction method by MDR

software3.0.2 was used to construct gene–gene interaction or gene–environment interaction. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. *P* is significant if ≤ 0.05 at confidence interval 95 %.

Results

Characteristics of the Study Population

The demographic, laboratory, clinical, and radiologic characteristics of patients and control groups are shown in Table 1. Except for AFP level, there was a non-significant difference in distribution of smoking index and also in the median TLC, hemoglobin, platelets, albumin, bilirubin, ALT, AST, and INR between the HCC and liver cirrhosis patients. On the other hand, these parameters except for TLC, INR, and ALT were significantly different between each of HCC and liver cirrhosis patients as compared to control group.

Among HCC patients, 27 cases (67.5 %) had tumor size ≥ 3 cm and 15 (37.5 %) had multiple tumor masses. The number and percentages of HCC patients with BCLC stages A, B, C, and D were 18(45 %), 10(25 %), 6(15 %), and 6(15 %), respectively. The median follow-up time was 12 months (ranging from 2 to 18 months). By the last follow-up, 88.6 % of patients were dead, Cumulative Overall Survival (proportion surviving at 12 months) was 11.4 %, and the mean survival time for the entire cohort of patients was 6.664 (5.198–8.131) months (data not shown in the table).

Table 2 reports the distribution of the polymorphisms in *CYP1A1* and *GSTP1* among HCC, cirrhotic patients, and controls. This sample of individuals was

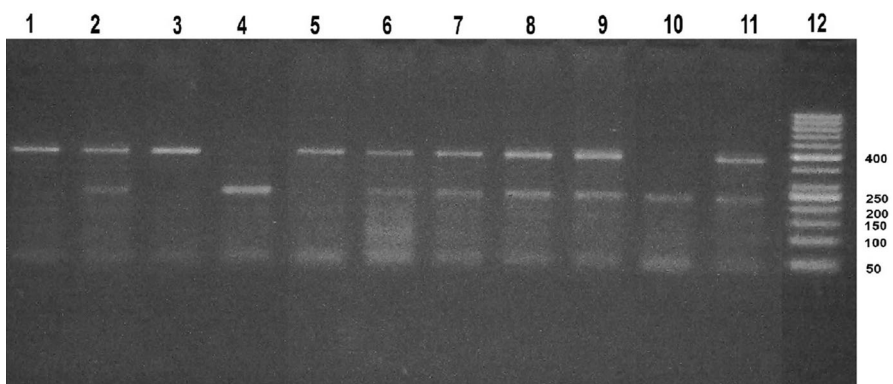


Fig. 2 Agarose gel electrophoresis stained with ethidium bromide. Showing bands of GSTP1 gene digested PCR product with *BsmA1* restriction enzyme using 50 bp ladder marker (Lane 12), showing wild type (*Ile/Ile*) with fragment length 440 bp (Lanes 1, 3 and 5); heterozygous (*Ile/Val*) with fragments 440, 212, and 228 bp (Lanes 2, 6, 7, 8, 9, and 11); and homozygous (*Val/Val*) with fragments 212 and 228 bp (Lanes 4 and 10). NB: Fragments 212 and 228 are overlapped in this figure

selected randomly, but they were unrelated. Applying HWE revealed that *GSTP1* and *CYP1A1* genotypes in LC, HCC cases and control subjects were independent (i.e., they are in HWE). There is no evidence to reject the assumption of HWE in the sample ($P > 0.05$ for each). The frequencies of *Ile/Val* genotype and *Val* allele are more common among HCC (37.5 and 23.8 %) than cirrhotic patients (15 and 10 %) corresponding to an OR of 2.2 and 2.8 (95 % CI 1.14–4.26 and 1.15–6.85) after correction for age and gender and to an OR of 2.18 and 1.65 (95 % CI 1.12–4.24 and 1.05–2.61) after bonferroni correction. So, *Ile/Val* genotype was risky for development of HCC within LC patients even after adjustment for age and sex.

On the other hand, the frequency of the variant genotypes m1 and m3 of *CYP1A1* was not significantly different between studied groups. The selected polymorphisms of *CYP1A1* cannot be considered as a risk for HCC development. Since the allelic frequencies for m1 and m3 were relatively of low numbers, we combined *m1* and *m3* together. The percentage of patients carrying *CYP1A1* wild-type gene among HCC and cirrhotic patients were 77.5 % (31/40) and 87.5 % (35/40), respectively.

Table 1 Demographic, laboratory, clinical, and radiologic data of studied groups

Data	Control group	LC group	HCC group	<i>P1</i>	<i>P2</i>	<i>P3</i>
Age (years)	54.80 ± 6.28	57.63 ± 7.27	55.58 ± 7.90	0.216	0.701	0.164
Males	30 (75 %)	29 (72.5 %)	32 (80 %)	0.592	0.799	0.836
Smoker	10 (25 %)	21 (52.5 %)	20 (50 %)	0.823	0.021*	0.012*
Smoking index (pack/year)	30 (20–35)	42.5 (30–94)	33.5 (22–105)	0.059	0.017*	<0.001*
Leucocytic count ($\times 10^9/l$)	5.9 (4.6–8)	4.8 (1.9–16)	5 (2–11)	0.311	0.126	0.138
Hemoglobin g/dl	13.2 (12.6–15.5)	9.1 (6–4)	9.7 (7–16)	0.765	<0.001*	<0.001*
Platelets ($\times 10^9/l$)	230 (170–340)	68 (24–196)	86 (32–266)	0.227	<0.001*	<0.001*
INR	1 (0.9–1.1)	1.2 (1–1.6)	1.3 (0.9–2)	0.082	0.104	0.101
Albumin g/dl	4.25 (3.6–4.9)	2.8 (1.7–3.8)	3 (2–4)	0.115	<0.001*	<0.001*
Bilirubin mg/dl	0.8 (0.7–1)	2.4 (1.1–7.2)	3 (1–5.3)	0.168	<0.001*	<0.001*
ALT (U/ml)	28.5 (18–42)	29.5 (17–77)	32 (8–108)	0.122	0.091	0.730
AST (U/ml)	28.5 (19–45)	52 (15–110)	52 (13–145)	0.422	0.028*	0.041*
AFP (ng/ml)	5.2 (2.4–9.5)	5.25 (2.1–16)	116.5 (1.8–4000)	<0.001*	<0.001*	0.869
Child-Pugh A	–	8 (20)	16 (40)	0.119	–	–
Child-Pugh B	–	25 (62.5)	17 (42.5)			
Child-Pugh C	–	7 (17.5)	7 (17.5)			

Data are expressed as median (range) except age as mean ± SD, gender, smokers, and Child-Pugh score as number (%)

P1, comparison between HCC gr. versus LC gr; *P2*, comparison between HCC gr. versus LC gr; *P3*, comparison between HCC gr. versus control gr; *P3*, comparison between LC gr. versus control gr; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR; international normalized ratio

Table 2 Distribution of alleles and genotypes in studied groups

Genotypes and alleles	Control group N (%)	LC group N (%)	HCC group N (%)	P1 P2	OR1 (95 % CI) OR2 (95 % CI)	P1 [†] P2 [†]	P1 [†] C P2 [†] C	OR1 [†] C (195 % CI) OR2 [†] C (95 % CI)
Ile/Ile	31 (77.5)	33 (82.5)	23 (57.5)	Reference	Reference	Reference	Reference	Reference
GSTP1								
Ile/Val	8 (20)	6 (15)	15 (37.5)	0.556	0.71 (0.22–2.26)	0.788	1.000	0.85 (0.25–2.89)
GSTP1				0.018*	2.21 (1.14–4.26)	0.022*	0.044*	2.18 (1.12–4.24)
Val/Val	1 (2.5)	1 (2.5)	2 (5)	0.965	0.94 (0.06–11.68)	0.888	1.000	1.23 (0.07–13.25)
GSTP1				0.392	1.93 (0.423–8.68)	0.425	0.850	1.84 (0.41–8.22)
Ile/Val+Val/Val	9 (22.5)	7 (17.5)	17 (42.5)	0.577	0.86 (0.49–1.48)	0.839	1.000	0.94 (0.53–1.69)
Ile allele	70 (87.5)	72 (90)	61 (76.3)	0.015*	2.17 (1.16–4.05)	0.019*	0.038*	2.13 (1.13–4.02)
Val allele	10 (12.5)	8 (10)	19 (23.8)	0.617	0.78 (0.29–2.09)	0.907	1.000	0.97 (0.58–1.62)
Wild CYP1A1	40 (100)	35 (87.5)	31 (77.5)	0.020*	2.80 (1.15–6.85)	0.031*	0.062	1.65 (1.05–2.61)
(m1+m3)				Reference	Reference	Reference	Reference	Reference
Variant CYP1A1	0 (0)	5 (12.5)	9 (22.5)	0.055	1.14 (1.02–1.29)	0.147	0.294	1.44 (0.88–2.35)
(m1+m3)				0.239	2.03 (0.62–6.72)	0.514	1.000	1.09 (0.84–1.41)

P1, comparison between LC versus control groups; P2, comparison between HCC and LC groups; 95 % CI, 95 % confidence interval; OR, odds ratio; C, corrected P value by Bonferroni correction

* P is significant

[†] Adjusted P value for age and gender

Table 3 Gene-smoking interaction among studied groups

Genotypes and alleles	Control group N (%)	LC group N (%)	HCC group N (%)	<i>P</i> ₁ <i>P</i> ₁ / <i>C</i>	OR1 (95 % CI)	<i>P</i> ₂ <i>P</i> ₂ / <i>C</i>	OR2 (95 % CI)
Wild <i>GSTP1</i> × non-smokers	27 (67.5)	16 (40)	11 (27.5)	Reference			
Wild <i>GSTP1</i> × smokers	4 (10)	17 (42.5)	12 (30)	0.002*	1.37 (1.05–12.09)	0.961	1.03 (0.35–2.98)
Variant <i>GSTP1</i> × non-smokers	3 (7.5)	3 (7.5)	9 (22.5)	0.004*	1.69 (0.30–9.38)	0.057	3.36 (0.96–13.86)
Variant <i>GSTP1</i> × smokers	6 (15)	4 (10)	8 (20)	1.000	1.13 (0.28–4.60)	0.114	2.91 (0.70–10.09)
Wild <i>CYP1A1</i> × non-smokers	30 (75)	16 (40)	16 (40)	Reference			
Wild <i>CYP1A1</i> × smokers	10 (25)	19 (47.5)	15 (37.5)	0.011	3.56 (1.34–9.46)	0.632	0.79 (0.30–2.08)
Variant <i>CYP1A1</i> × non-smokers	0 (0)	3 (7.5)	4 (10)	0.022*	1.48 (0.64–3.43)	0.732	1.33 (0.26–6.94)
Variant <i>CYP1A1</i> × smokers	0 (0)	2 (5)	5 (12.5)	0.444	1.48 (0.54–4.08)	0.313	2.50 (0.42–14.83)
				0.888		0.626	

Wild or variant *CYP1A1* = m1+m3

Variant *GSTP1* = *Ile/Val*+*Val/Val*

Wild *GSTP1* = *Ile/Ile*

*P*₁, comparison between LC and control groups; *P*₂, comparison between HCC and LC groups; OR, odds ratio; 95 % CI, 95 % confidence interval; C, corrected *P* value by Bonferroni correction

* *P* is significant

All control subjects and liver cirrhotic patients carry the wild *CYP1A1* genotype apart from 5 (12.5 %) of cirrhotic patients who carried m3 heterozygote's (Table 2). m1 heterozygous and m3 heterozygous genotypes were detected among 2(5 %) and 6(15 %) of patients with HCC. Only one patient (2.5 %) with m1/m1 variant (m1 homozygote) was detected among HCC patients (data not shown in the table).

The baseline demographic, laboratory, clinical, and radiologic data versus *CYP1A1* and *GSTP1* genotypes were studied in HCC patients. There were no differences between patients carrying wild and variant genotypes of *CYP1A1* and *GSTP1* except for age, as HCC patients with variant *CYP1A1* were significantly older than those with wild type (P , 0.026) with mean ages of 60.7 and 54.1, respectively, also *Ile/Val* and *Val/Val* genotypes were significantly associated with larger tumor size (P , 0.046) as 100 and 86.7 % of HCC patients with *Ile/Val* and *Val/Val* genotypes had tumor size larger than 3 cm. On the other hand, gender, Child-Pugh, BCLC scores, and overall survival were insignificantly different between variants and wild genotypes carrying HCC patients (data not shown in the tables).

Gene–smoking interaction analysis was conducted, using the wild-type homozygous, non-smokers as a reference group; while for gene–gene interaction analysis, the homozygous wild-type individuals for both genes were the reference group. Statistical analysis combining different genetic distributions for estimating the interaction effect between the *GSTP1* and *CYP1A1* gene polymorphisms on the susceptibility to HCC (Table 4), and also with smoking (Tables 3 and 5) was performed. MDR analyses were also performed and shown in Fig. 3 in the form of bars representing hypothetical distributions of multifactor combination of *CYP1A1*, *GSTP1*, and smoking in control subjects, LC and HCC patients in comparison with each other. High-risk genotype, smoking interaction with ratio greater than one was observed when *CYP1A1* mutants genotypes combined with smoking were compared between cirrhotic and control subjects or between cirrhotic and HCC subjects. For *GSTP1* and smoking combination, high risk was reported when *Val/Val* genotype with smoking was compared between cirrhotic and control subjects or between cirrhotic and HCC subjects, also the ratio is greater than one when *Ile/Val* genotype with smoking was compared between cirrhotic and HCC patients. No cases were observed to carry the combined *CYP1A1* and *GSTP1* mutation among control or cirrhotic subjects, while the combination between *Ile/Val* or *Val/Val* genotypes with *CYP1A1* mutants was risky as shown by the increase in the ratio between HCC and cirrhotic subjects. The only available data to test *CYP1A1*, *GSTP1* smoking interaction was between *CYP1A1* mutants, *Ile/Val* genotype and smoking which are risky between HCC and cirrhotic patients. In spite of this increase in patients to control ratio, there is no statistical significant increase in risk on combining both variants (Table 4) and also on combining both variants with smoking (Table 5). Smoking in the presence of *Ile/Ile* genotype was considered a risk factor for LC development (P , 0.004) with OR of 1.37 and 95 % CI of 1.05–12.09. The same findings are shown in the presence of smoking with wild *CYP1A1* (P , 0.022) with OR of 3.56 and 95 % CI of 1.34–9.46 which is attributed to smoking (Table 3).

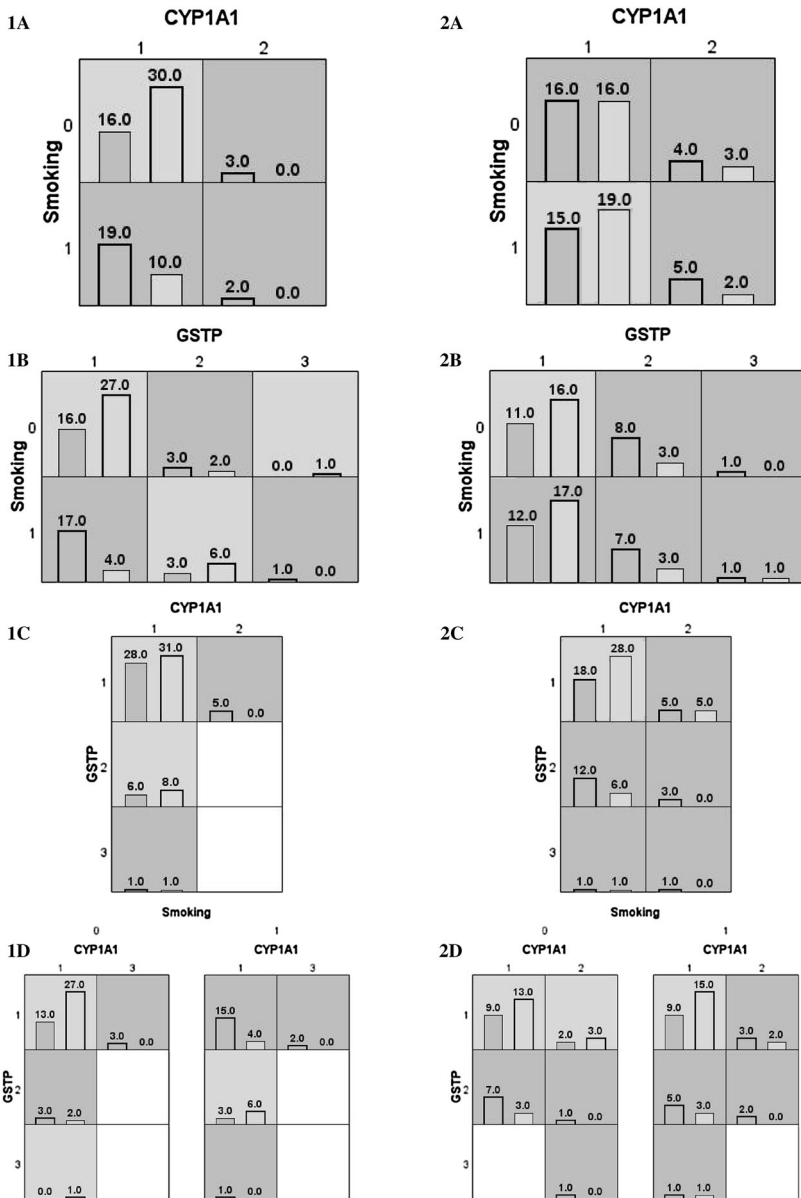


Fig. 3 MDR analysis for gene–gene and gene–environmental interaction. Bars represent hypothetical distributions of (1) control (left) and LC (right); (2) LC (left) and HCC (right) with each multifactor combination. Dark shaded cells represent genotype combinations with increased odds ratio, while light shaded cells represent genotype combinations with decreased odds ratio. No shading or white cells represent genotype combinations for which no data were observed. Smoking is divided into 0 (non-smoker) and 1 (smoker); *CYP1A1* is divided into 1 (wild) and 2 (variant); *GSTP1* is divided into 1 (*II/II*), 2 (*II/Val*) and 3 (*Val/Val*)

Table 4 Interaction of *GSTP1* and *CYP1A1* genes among studied groups

<i>GSTP1</i> × <i>CYP1A1</i>	Control group <i>N</i> (%)		LC group <i>N</i> (%)		HCC group <i>N</i> (%)		<i>P1</i>		<i>P2</i>		<i>P1</i> C		<i>P2</i> C			
	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>P1</i>	<i>P2</i>	OR1	95 % CI	OR2	95 % CI	OR1 C	95 % CI	OR2 C	95 % CI
Wild <i>GSTP1</i> × wild <i>CYP1A1</i>	31	(77.5)	28	(70)	18	(45)	Reference	Reference								
Wild <i>GSTP1</i> × variant <i>CYP1A1</i>	0	(0)	5	(12.5)	5	(12.5)	0.361	0.361	1.36	(0.71–2.61)	1.36	(0.71–2.61)	0.307	0.307	1.44	(0.72–2.89)
Variant <i>GSTP1</i> × wild <i>CYP1A1</i>	9	(22.5)	7	(17.5)	13	(32.5)	0.841	0.841	1.05	(0.68–1.61)	1.05	(0.68–1.61)	0.823	0.823	1.05	(0.68–1.634)
Variant <i>GSTP1</i> × variant <i>CYP1A1</i>	0	(0)	0	(0)	4	(10)	0.539	0.539	1.11	(0.80–1.54)	1.11	(0.80–1.54)	0.574	0.574	1.10	(0.79–1.53)
							NA	0.456	NA	1.26	NA	1.26	NA	NA	1.29	NA
																0.420
																(0.70–2.38)

Variant *GSTP1* = *Ile/Val* + *Val/Val*

Wild *GSTP1* = *Ile/Ile*

NA = not applicable, OR = odds ratio; 95 % CI, 95 % confidence interval; *P1*, comparison between LC and control groups; *P2*, comparison between HCC and LC group; C, *P* value adjusted for age and gender

Table 5 Smoking *GSTP1*, *CYP1A1* genes interaction and risk of HCC

<i>CYP1A1</i> × <i>GSTP1</i>	Smoking	LC group <i>N</i> (%)	HCC group <i>N</i> (%)	<i>P</i>	OR (95 % CI)
Wild <i>CYP1A1</i> × wild <i>GSTP1</i>	No	13 (32.5)	9 (22.5)	Reference	
Wild <i>CYP1A1</i> × wild <i>GSTP1</i>	Yes	15 (37.5)	9 (22.5)	0.940	0.99 (0.68–1.43)
Wild <i>CYP1A1</i> × variant <i>GSTP1</i>	No	3 (7.5)	7 (17.5)	0.630	1.12 (0.71–1.78)
Wild <i>CYP1A1</i> × variant <i>GSTP1</i>	Yes	4 (10)	6 (15)	0.750	1.08 (0.68–1.73)
Variante <i>CYP1A1</i> × wild <i>GSTP1</i>	No	3 (7.5)	2 (5)	0.991	0.99 (0.53–1.86)
Variante <i>CYP1A1</i> × wild <i>GSTP1</i>	Yes	2 (5)	3 (7.5)	0.805	1.08 (0.59–1.98)
Variante <i>CYP1A1</i> × variante <i>GSTP1</i>	No	0 (0)	2 (5)	0.611	1.25 (0.54–2.90)
Variante <i>CYP1A1</i> × variante <i>GSTP1</i>	Yes	0 (0)	2 (5)	0.611	1.25 (0.54–2.90)

Variante *GSTP1* = *Ile/Val+Val/Val*

Wild *GSTP1* = *Ile/Ile*

OR, odds ratio; 95 % CI, 95 % confidence interval

Discussion

Most environmental carcinogens require prior metabolic activation in order to elicit their effects. Many of the enzymes involved in carcinogen metabolism exhibit genetic polymorphisms resulting in variability in enzyme activity (Salaspuro and Salaspuro 2004).

This study analyzed the influence of smoking as well as *CYP1A1*, *GSTP1* polymorphic variants, and their interaction on the development of HCV-related HCC. The number of current or former smokers among HCC and cirrhotic patients as well as the median Pack/year of cigarette smoked was significantly higher in HCC and liver cirrhotic patients than in control group (Table 1). The most important finding was that individuals carrying the *Ile/Val* genotype had a significant increased risk of HCC (Table 2). This finding is consistent with previous reports demonstrating *GSTP1* polymorphisms as a contributing factor to the increased risk and poor outcome of many cancers, including esophageal cancer (Li et al. 2010), breast cancer (Yang et al. 2005), and HCC in Taiwanese patients younger than 57 years (Chen et al. 2010). It was also observed that altered expression of *GSTP1* existed in liver cancer cell lines (Ding et al. 2004), and in more than 77.8 % of HBV-associated HCC tissues (Zhong et al. 2002). Li et al. (2012) indicated carriage of *GSTP1* Val/Val genotypes to have roles in susceptibility to HCC with OR (95 % CI) of 1.93 (1.32–2.95). Moreover, Li et al. (2013) found that *GSTP1* protein and mRNA expression in HCC patients were significantly decreased when compared to chronic hepatitis B (CHB) patients. *GST* family acts as

a part of the protection system against a wide range of potentially harmful cytotoxic compounds. Therefore, our results may support the idea that *GSTP1* variants are associated with decreased *GSTP1* activity that takes part in the development of HCC following HCV infection which is accompanied by massive ROS production.

The present study reported no increased risk of development of cirrhosis ($P = 0.110$) with *CYP1A1* gene variants (m1 and m3) and did not show significant association ($P = 0.478$) with risk of HCC development among cirrhotic patients (Table 2).

In accordance with this, Boccia et al. (2015) reported that *CYP1A1 MspI* polymorphism did not significantly influence susceptibility to HCC. However, Silvestri et al. (2003) reported that the frequencies of the *CYP1A1* highly inducible alleles, *MspI* m2 and *Val*, were increased in HCV-related liver disease compared with healthy subjects, in particular with asymptomatic carriers. No association with HCC was found, and no relationship with tobacco smoke was seen. Similar observation was obtained in previous studies on hepatitis B carriers (Yu et al. 1999, 2015). Several studies have suggested the *CYP1A1* polymorphisms to be associated with elevated risks of prostate cancer (Ding et al. 2013), esophageal cancer (Gong et al. 2014), and head and neck cancer (Liu et al. 2013). However, no significant associations between the *CYP1A1* polymorphisms and risks for gastric cancer (And and Guo 2012) and colorectal cancer were found (Zheng et al. 2012).

The contradictory findings among different studies may be explained by the difference in ethnicity or by the possibility that polymorphisms of *CYP1A1* might exert different effects in different types of tissues, an idea that needs to be confirmed. Our results revealed that the mean age of patients with *CYP1A1* variants (60.7 years) was higher than those with wild *CYP1A1* type (54.1 years) and so these variants may evoke their effect among elderly individuals.

In the current study, we estimated the effect of these *CYP1A1* and *GSTP1* gene polymorphisms on the clinical status, such as clinical stage, tumor size, Child-Pugh grade, overall survival (OS), and the serum levels of liver-related biochemical markers, such as alpha-fetoprotein, AST, and ALT in HCC patients. Except for tumor size with *GSTP1*, a lack of association between the gene polymorphisms and those estimated factors was found. This study shows that HCC patients with *GSTP1* variants had larger tumor size than patients with wild type ($P = 0.046$). Similar results were reported previously (Chen et al. 2010).

Analyses of gene–gene interactions in relation to susceptibility to tobacco carcinogens were carried out. In this study, there is an increased ratio of number of smokers with *CYP1A1* mutation or *Val/Val* genotype of *GSTP1* in cirrhotic than control subjects and in HCC than cirrhotic patients. The ratio of smokers with *Ile/Val* genotype of *GSTP1* was also higher in HCC patients than cirrhotic patients. Moreover, the combination between *Ile/Val* or *Val/Val* genotypes of *GSTP1* with *CYP1A1* mutants or between *CYP1A1* mutants, *Ile/Val* genotype and smoking was risky as shown by the increased ratio between cirrhotic and HCC subjects (Fig. 3). In spite of this increase in patients to control ratio, there are no statistically significant synergistic gene–gene, gene smoking or gene–gene–smoking interactions in association to HCC development (Tables 4 and 5). Similar results were obtained with lung cancer by previous authors (López-Cima et al. 2012). However,

Boccia et al. (2015) observed a borderline increased risk for HCC among carriers of combined *CYP1A1* and *SULT1A1* variant alleles as compared to the double wild-type homozygous. To the best of our knowledge, this is the first study to analyze the role of interaction of *CYP1A1* and *GSTP1* gene polymorphism as a risk for HCC among Egyptian patients chronically infected with HCV.

The cumulative overall survival (OS) in HCC patients included in this study was analyzed with the possible role of polymorphic *CYP1A1* and *GSTP1* in determining survival outcome (Fig. 4). No significant differences were found in OS between

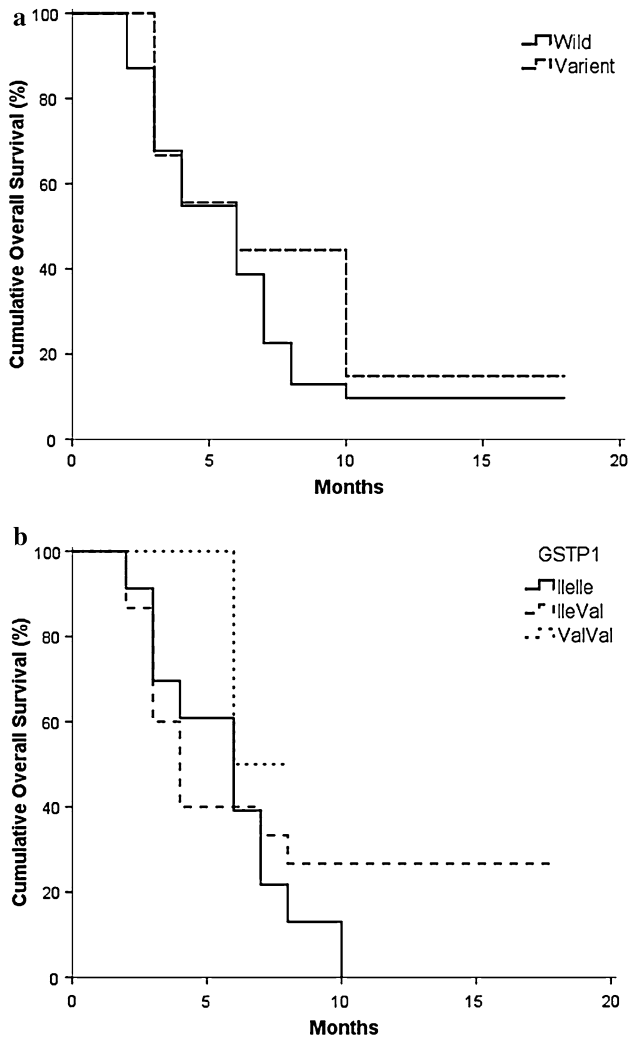


Fig. 4 Kaplan-Meier curves for hepatocellular carcinoma patients carrying genetic variants of *CYP1A1* rs7085725 (a) and *GSTP1*: rs4147581 (b). No significant differences were found in OS between *CYP1A1* as well as *GSTP1* genotypes. ($P = 0.32$ and 0.38 , respectively)

HCC patients carrying wild and variant *CYP1A1* as well as *GSTP1* genotypes ($P = 0.32$ and 0.38 , respectively). Contrary to this finding, Li et al. (2012) found that *GSTP1* Ile/Val or *GSTP1* Val/Val genotypes had better survival outcomes. Also Qu et al. (2015) found that patients with *GSTP1*, WV+VV genotypes of SNP rs4147581 had a longer median survival time as compared to those with WW genotype. It is to be noted that the age, sex, smoking behavior, liver cirrhosis, Child-Pugh score, AFP, and tumor size differ among these studies.

Conclusion

This study shows that the risk of developing HCC in Egyptian population can be partly explained by genetic polymorphisms in *GSTP1* gene. On the other hand, no significant increase in risk of HCC development was noted with *CYP1A1* m1 and m3 gene variants. Both genes variants have no association with survival. No statistically significant gene–gene or gene–smoking interaction was reported in association to HCC risk with only increase in ratio of combined *CYP1A1* gene mutants, *GSTP1* *II/Val* and smoking which need extension of the study on large number of patients.

Acknowledgments The authors thank Dr. Iman Fawzy (MD) for performing the statistical analysis.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

- Allan JM, Wild CP, Rollinson S, Willett EV, Moorman AV, Dovey GJ, Roddam PL, Roman E, Cartwright RA, Morgan GJ (2001) Polymorphism in Glutathione *S*-transferase P1 is associated with susceptibility to chemotherapy—induced leukemia. *Proc Natl Acad Sci USA* 98:11592–11597
- And GR, Guo X (2012) Quantitative assessment of the associations between *CYP1A1* polymorphisms and gastric cancer risk. *Tumor Biol* 33:1125–1132
- Boccia S, Miele L, Panic N, Turati F, Arzani D, Cefalo C, Amore R, Bulajic M, Pompili M, Rapaccini G, Gasbarrini A, La Vecchia C, Grieco A (2015) The effect of CYP, GST, and SULT polymorphisms and their interaction with smoking on the risk of hepatocellular carcinoma. Hindawi Publishing Corporation. *Bio Med Research International*, Article ID 179867, 7 pages
- Cascorbi I, Brockmoller J, Roots I (1996) A C4887A polymorphism in exon 7 of human *CYP1A1*: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res* 56:4965–4969
- Chen YL, Tseng HS, Kuo WH, Yang SF, Chen DR, Tsai HT (2010) Glutathione *S*-transferase P1 (*GSTP1*) gene polymorphism increases age-related susceptibility to hepatocellular carcinoma. *BMC Med Genet* 11:46
- Cholongitas E, Papatheodoridis GV, Vangeli M, Terreni N, Patch D, Burroughs AK (2005) Systematic review: the model for end-stage liver disease—should it replace Child-Pugh’s classification for assessing prognosis in cirrhosis? *Aliment Pharmacol Ther* 22(11–12):1079–1089
- Ding S, Gong BD, Yu J, Gu J, Zhang HY, Shang ZB, Fei Q, Wang P, Zhu JD (2004) Methylation profile of the promoter CpG islands of 14 “drug-resistance” genes in hepatocellular carcinoma. *World J Gastroenterol* 10:3433–3440

- Ding G, Xu W, Liu H, Zhang M, Huang Q, Liao Z (2013) *CYP1A1* MspI polymorphism is associated with prostate cancer susceptibility: evidence from a meta-analysis. *Mol Biol Rep* 40:3483–3491
- El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 132:2557–2576
- El-Zayadi AR, Badran HM, Barakat EM, Mel-D A, Shawky S, Mohamed MK, Selim O, Saied A (2005) Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J Gastroenterol* 11:5193–5198
- Forner A, Gilibert M, Bruix J, Raoul JL (2014) Treatment of intermediate-stage hepatocellular carcinoma. *Nat Rev Clin Oncol* 11(9):525–535
- Gong FF, Lu SS, Hu CY, Qian ZZ, Feng F, Wu YL, Yang HY, Sun YH (2014) Cytochrome P450 1A1 (*CYP1A1*) polymorphism and susceptibility to esophageal cancer: an updated meta-analysis of 27 studies. *Tumor Biol* 35:10351–10361
- Gross-Steinmeyer K, Eaton DL (2012) Dietary modulation of the biotransformation and genotoxicity of aflatoxin B (1). *Toxicology* 299:69–79
- Hengstler JG, Arand M, Herrero ME, Oesch F (1998) Polymorphisms of *N*-acetyltransferases, glutathione *S*-transferases, microsomal epoxide hydrolase and sulfotransferases: influence on cancer susceptibility. *Eur Pub Med Central* 154:47–85
- Huang S, Wu F, Luo M, Ma L, Gao K, Li J, Wu W, Huang S, Yang Q, Liu K, Zhao Y, Li L (2013) The glutathione *S*-transferase P1 341C > T polymorphism and cancer risk: a meta-analysis of 28 case-control studies. *PLOS One* 8(2):e56722
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. *CA Cancer J Clin* 61:69–90
- Kato T, Yamano Y, Tsuji M, Watanabe M (2008) Genetic polymorphisms of human cytosol glutathione *S*-transferases and prostate cancer. *Pharmacogenomics* 9:93–104
- Kweekel DM, Koopman M, Antonini NF, Van der Straaten T, Nortier JW, Gelderblom H, Punt CJ, Guchelaar HJ (2008) *GSTP1* Ile105Val poly-morphism correlates with progression-free survival in MCRC patients treated with or without irinotecan: a study of the Dutch colorectal cancer group. *Br J Cancer* 99:1316–1321
- Li R, Shugart YY, Zhou W, An Y, Yang Y, Zhou Y, Zhang B, Lu D, Wang H, Qian J, Jin L (2009) Common genetic variations of the cytochrome P450 1A1 gene and risk of hepatocellular carcinoma in a Chinese population. *Eur J Cancer* 45:1239–1247
- Li D, Dandara C, Parker MI (2010) Ther341C/T polymorphism in the *GSTP1* gene is associated with increased risk of oesophageal cancer. *BMC Genet* 11:47
- Li CG, Zhao ZM, Hu MG, Liu R (2012) Predictive role of glutathione-*S*-transferase gene polymorphisms in risk and prognosis of hepatocellular carcinoma. *Asian Pacific J Cancer Prev* 13:3247–3252
- Li T, Zhao X, Wang L, Gao S, Zhao J, Fan Y, Wang K (2013) Glutathione *S*-transferase P1 correlated with oxidative stress in hepatocellular carcinoma. *Int J Med Sci* 10(6):683–690
- Liu L, Wu G, Xue F, Li Y, Shi J, Han J, Zhang X, Na Y, Zhang H, Tang X, Pu H, Yuan Q, Zhang L, Yang M (2013) Functional *CYP1A1* genetic variants, alone and in combination with smoking, contribute to development of head and neck cancers. *Eur J Cancer* 49:2143–2151
- López-Cima MF, Álvarez-Avellón SM, Pascual T, Somoano AF, Tardón A (2012) Genetic polymorphisms in *CYP1A1*, *GSTM1*, *GSTP1* and *GSTT1* metabolic genes and risk of lung cancer in Asturias. *BMC Cancer* 12:433
- National Research Council (1983) Polycyclic aromatic hydrocarbons: evaluation of sources and effects. National Academy Press, Washington, pp 197–262
- Nerurkar PV, Okinaka L, Aoki C, Seifried A, Lum-Jones A, Wilkens LR, Le Marchand L (2000) *CYP1A1*, *GSTM1*, and *GSTP1* genetic polymorphisms and urinary 1-hydroxypyrene excretion in non-occupationally exposed individuals. *Cancer Epidemiol Biomark Prev* 9:1119–1122
- Qu K, Liu S, Wang Z, Huang Z, Liu S, Chang H, Xu X, Lin T, Dong Y, Liu C (2015) Polymorphisms of glutathione *S*-transferase genes and survival of resected hepatocellular carcinoma patients. *World J Gastroenterol* 21(14):4310–4322
- Ryder SD (2003) Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut* 52(suppl 3):1111–1118
- Salaspuo V, Salaspuo M (2004) Synergistic effect of alcohol drinking and smoking on in vivo acetaldehyde concentration in saliva. *Int J Cancer* 111:480–483
- Silvestri L, Sonzogni L, De Silvestri A, Grizzi C, Foti L, Zavaglia C, Leverì M, Cividini A, Mondelli MU, Civardi E, Silini EM (2003) CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer. *Int J Cancer* 104(3):310–317

- Smith G, Stanley LA, Sim E, Strange RC, Wolf CR (1995) Metabolic polymorphisms and cancer susceptibility. *Cancer Surv* 25:27–65
- Yang G, Shu XO, Ruan ZX, Cai QY, Jin F, Gao YT, Zheng W (2005) Genetic polymorphisms in glutathione-S-transferase genes (*GSTM1*, *GSTT1*, *GSTP1*) and survival after chemotherapy for invasive breast carcinoma. *Cancer* 103:52–58
- Yu MW, Chiu YH, Yang SY, Santella RM, Chern HD, Liaw YF, Chen CJ (1999) Cytochrome P450 1A1 genetic polymorphisms and risk of hepatocellular carcinoma among chronic hepatitis B carriers. *Br J Cancer* 80(3–4):598–603
- Yu BW, Zhang LQ, Teng XL, Zhang Y, Zou LB, Ying HY (2015) Association between the *CYP1A1* polymorphisms and hepatocellular carcinoma: a meta-analysis. *Genet Mol Res* 14(1):1076–1084
- Zheng Y, Wang JJ, Sun L, Li HL (2012) Association between *CYP1A1* polymorphism and colorectal cancer risk: a meta-analysis. *Mol Biol Rep* 39:3533–3540
- Zhong S, Tang MW, Yeo W, Liu C, Lo YM, Johnson PJ (2002) Silencing of *GSTP1* gene by CpG island DNA hypermethylation in HBV associated hepatocellular carcinomas. *Clin Cancer Res* 8:1087–1092