The Effect of CYP, GST, and SULT Polymorphisms and Their Interaction with Smoking on the Risk of Hepatocellular Carcinoma

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Received 16 April 2014; Revised 19 June 2014; Accepted 19 June 2014

Academic Editor: Paolo Boffetta

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Aim. The aim of our study was to assess whether selected single nucleotide polymorphisms of CYPIA1 and 2E1, GSTM1, GSTT1, and SULT1A1 influence susceptibility towards HCC, considering their interaction with cigarette smoking. Methods. We recruited HCC cases and controls among patients admitted to the hospital "Agostino Gemelli," from January 2005 until July 2010. Odds ratios (OR) of HCC were derived from unconditional multiple logistic regression. Gene-gene and gene-smoking interaction were quantified by computing the attributable proportion (AP) due to biological interaction. Results. The presence of any CYP2E1∗5B variant allele (OR: 0.23; 95% CI: 0.06–0.71) and CYP2E1∗6 variant allele (OR: 0.08; 95% CI: 0.01–0.33) was inversely related to HCC. There was a borderline increased risk among carriers of combined CYPIA1∗2A and SULT1A1 variant alleles (OR: 1.67; 95% CI: 0.97–3.24). A significant biological interaction was observed between GSTT1 and smoking (AP = 0.48; 95% CI: 0.001–0.815), with an OR of 3.13 (95% CI: 1.69–5.82), and borderline significant interaction was observed for SULT1A1 and smoking (AP = 0.36; 95% CI: −0.021–0.747), with an OR of 3.05 (95% CI: 1.73–5.40). Conclusion. CYP2E1∗5B and CYP2E1∗6 polymorphisms have a favourable effect on the development of HCC, while polymorphisms of GSTT1 and SULT1A1 might play role in increasing the susceptibility among smokers.

1. Introduction

Hepatocellular carcinoma (HCC) is currently the sixth most common cancer and the third cause of cancer deaths worldwide [1]. Its prognosis remains poor, with a 5-year survival rate less than 20% in Europe [2]. Risk factors for HCC include infection with hepatitis B (HBV) and hepatitis C viruses (HCV), history of diabetes mellitus, nonalcoholic fatty liver disease and cirrhosis, heavy alcohol consumption, and cigarette smoking [3–5]. Coffee consumption appears to have a favorable effect [6]. Further, genetic factors appear to modulate the individual susceptibility as the siblings of HCC
individuals are more prone to develop the HCC even in the absence of HBV infection [7]. So far, several single nucleotide polymorphisms (SNPs) have been investigated in association with HCC, with contradictory results [8].

As the liver is the main metabolic organ, the SNPs related to genes encoding carcinogen-metabolizing enzymes represent key target candidates for association analyses. Cytochrome P-450 (CYP) is a superfamily of monooxygenases responsible for phase I enzyme reactions, preparing substrates for phase II conjugation reactions, but they can also lead to the metabolic activation of toxic or carcinogenic compounds [9]. The glutathione S-transferases (GSTs) are a gene superfamily coding phase II enzymes that detoxify free radicals in tobacco smoke, products of oxidative stress, and polycyclic aromatic hydrocarbons [10]. Sulfotransferase (SULT) catalyzes sulfonate conjugation to detoxify the procarcinogens to metabolites, which are easily eliminated from the body [11]. Polymorphisms in these genes, their combination, and interaction with environmental factors have the potential to lead to increased susceptibility to HCC. While some studies investigated the effect of GST and CYP genes on HCC, as well as their combination and interaction with smoking [12, 13], little information is available on the effect of SULT1A1.

The aim of our study was to assess whether the selected SNPs of CYP1A1 and 2E1, GSTM1, GSTT1, and SULT1A1 genes influence individual susceptibility to HCC, also considering their combination and interaction with cigarette smoking.

2. Methods

2.1. Study Population. Study participants were recruited among patients admitted to the teaching hospital "Agostino Gemelli" of the Università Cattolica del Sacro Cuore (Rome, Italy) from January 2005 until July 2010, and eligibility was restricted to white individuals born in Italy. Cases were 221 Italy) from January 2005 until July 2010, and eligibility was restricted to white individuals born in Italy. Cases were 221 cases of hepatocellular carcinoma (HCC) and 290 controls according to selected factors.

| HCC cases (N = 221) | Controls (N = 290) |
|---------------------|-------------------|
| Age (years)         |                   |
| <60                 | 43 (19.5)         | 105 (36.2) |
| 60–69               | 80 (36.2)         | 78 (26.9)  |
| ≥70                 | 98 (44.3)         | 107 (36.9) |
| Sex                 |                   |
| Male                | 160 (72.4)        | 176 (60.7) |
| Female              | 61 (27.6)         | 114 (39.3) |
| Smoking             |                   |
| Never               | 85 (39.2)         | 173 (59.7) |
| Ever                | 133 (60.8)        | 117 (40.3) |
| P< 0.001            |                   |
| Hepatitis           |                   |
| No                  | 64 (29.0)         | 282 (97.9) |
| Yes                 | 157 (71.0)        | 6 (2.1)    |
| P< 0.001            |                   |

The sum does not add up to the total because of some missing values.

P values from χ² test.

Hepatitis was defined as history of hepatitis B and/or C.

2.2. Genotyping Methods. DNA was extracted from the peripheral blood lymphocytes of each participating subject. GSTM1 and GSTT1 null alleles were identified using a multiplex-polymerase chain reaction- (PCR-) based method [15]. The polymorphic site at nucleotide 638 in exon 7 (Arg213His (∗2 allele), rs9282861) of the SULT1A1 gene was genotyped by PCR-restriction fragment length polymorphisms (RFLP) analysis as described by Cougthrie et al. [16]. CYP1A1 3′-flanking region MspI polymorphism (CYP1A1∗2A allele, rs4646903), CYP2E1 PstI/RsaI polymorphism (CYP2E1∗5B allele, rs3813867 (PstI)), and CYP2E1 DraI (∗5A or ∗6 alleles, rs6413432) were also determined by PCR-RFLP analyses. Quality control for each genotyping was performed in each experiment, and 10% of the total samples were randomly selected and reanalyzed with 100% concordance. All laboratory procedures were carried out blindly to case-control status.

2.3. Statistical Analysis. Hardy-Weinberg equilibrium (HWE) was tested for the control SNPs. Odds ratios (OR) of HCC and corresponding 95% confidence intervals (CI) according to analyzed polymorphisms were derived from unconditional multiple logistic regression models [17] using dominant model for carriers of the mutated allele, including terms for age and sex. When cell sizes were small (<5), exact logistic regression was used [18].

We also examined the possible confounding effect of smoking, alcohol, and chronic infection with HBV and/or HCV. However, models including these covariates yielded very similar results; thus, given the small numbers in some strata, only the age- and sex-adjusted estimates were presented.
Table 2: Distribution of cases and controls, odds ratios (OR), and 95% confidence intervals (CI) for hepatocellular carcinoma (HCC) according to selected polymorphisms.

|                | HCC cases (N = 221) | Controls (N = 290) | OR (95% CI) |
|----------------|---------------------|--------------------|-------------|
|                | N       | (%)    | N       | (%)    |           |
| CYP1A1<sup>∗</sup>2A |         |        |         |        |           |
| wt/wt          | 165     | (74.7) | 226     | (77.9) | 1<sup>b</sup> |
| wt/mt and mt/mt| 56      | (25.3) | 64      | (22.7) | 1.21 (0.80–1.84) |
| CYP2E1<sup>∗</sup>5B |         |        |         |        |           |
| c1/c1          | 217     | (98.2) | 270     | (93.1) | 1<sup>b</sup> |
| c1/c2 and c2/c2| 4       | (1.8)  | 20      | (6.9)  | 0.23 (0.06–0.71) |
| CYP2E1<sup>∗</sup>6<sup>c</sup> |         |        |         |        |           |
| wt/wt          | 204     | (99.0) | 261     | (90.0) | 1<sup>d</sup> |
| wt/mt and mt/mt| 2       | (1.0)  | 29      | (10.0) | 0.08 (0.01–0.33) |
| GSTM1<sup>f</sup> |         |        |         |        |           |
| Present        | 96      | (47.8) | 139     | (48.1) | 1<sup>b</sup> |
| Null           | 105     | (52.2) | 150     | (51.9) | 0.99 (0.68–1.43) |
| GSTT1<sup>f</sup> |         |        |         |        |           |
| Present        | 141     | (70.1) | 220     | (76.1) | 1<sup>b</sup> |
| Null           | 60      | (29.9) | 69      | (23.9) | 1.35 (0.89–2.05) |
| SULT1A1        |         |        |         |        |           |
| wt/wt          | 132     | (59.7) | 180     | (62.1) | 1<sup>b</sup> |
| wt/mt and mt/mt| 89      | (40.3) | 110     | (37.9) | 1.22 (0.84–1.77) |

<sup>a</sup>Adjusted for age and sex.
<sup>b</sup>Reference category.
<sup>c</sup>The sum does not add up to the total because of some missing values.
<sup>d</sup>Calculated from exact logistic regression analysis.

Gene-gene interaction analysis was conducted, using as a reference group the homozygous wild-type individuals for both genes, while for gene-environment interaction analyses, the reference group was wild-type homozygotes not exposed to the environmental risk factor. Biological interaction between two genes was estimated using departure from additivity of effects as the criterion of interaction, as proposed by Rothman [19]. To quantify the amount of interaction, the attributable proportion (AP) due to interaction was calculated together with its 95% CI as described by Andersson et al. [20]. The AP due to interaction is the proportion of individuals among those exposed to the two interacting factors that is attributable to the interaction per se and it is equal to 0 in the absence of a biological interaction [19]. In order to test for more than multiplicative effect among two genes, the likelihood ratio test was used.

The paper has been written according to the STREGA guidelines [21].

3. Results

The demographics, clinical features and lifestyle habits of 221 HCC cases and 290 controls are reported in Table 1. Ever smokers were more common among cases, as well as infection with HBV and/or HCV (Table 1). The genotype frequencies were in HWE (P > 0.05).

Table 2 reports the distribution of the polymorphisms considered among HCC patients and controls. The carriers of c2 variant allele of CYP2E1<sup>∗</sup>5B polymorphisms were less common in cases (1.8%) than in controls (6.9%) corresponding to an OR of 0.23 (95% CI: 0.06–0.71). Similarly, the frequency of the variant allele of CYP2E1<sup>∗</sup>6 polymorphism was also less common among cases (1.0%) than controls (10%), with an OR of 0.08 (95% CI: 0.01–0.33) (Table 2). The selected polymorphisms of CYP1A1, GSTM1, GSTT1, and SULT1A1 did not significantly influence susceptibility to HCC.

The gene-gene and gene-smoking interaction results are reported in Tables 3 and 4. We observed a borderline increased risk for HCC among carriers of combined CYP1A1<sup>∗</sup>2A and SULT1A1 variant alleles as compared to the double wild-type homozygotes (OR = 1.67; 95% CI: 0.97–3.24) (Table 3). A significant interaction was reported between GSTT1 and smoking (AP = 0.48; 95% CI: 0.001–0.815), with an OR of 3.13 (95% CI: 1.69–5.82) for GSTT1 null genotype carriers who were smokers (Table 4). A borderline significant interaction was also observed for SULT1A1 and smoking (AP = 0.36; 95% CI: −0.021–0.747), with an OR of 3.05 (95 CI: 1.73–5.40) for those SULT1A1 variant allele carriers who were smokers (Table 4).
### Table 3: Effect of the genes-gene interaction on the development of hepatocellular carcinoma.

| Interaction | Cases: controls | OR \(^a\) (95% CI) | \(P^b\) | AP (95% CI) |
|-------------|-----------------|----------------------|--------|-------------|
| **GSTM1 \(\times\) CYP1A1 \(^\ast\) 2A** |                     |                      |        |             |
| Present/wt homozygote | 74:113 | 1 \(^c\) |        |             |
| Null/wt homozygote | 78:112 | 1.05 (0.69–1.61) |        |             |
| Present/any mt | 22:26 | 1.32 (0.69–2.53) |        |             |
| Null/any mt | 27:38 | 1.04 (0.58–1.88) | 0.521 | nc          |
| **GSTM1 \(\times\) GSTT1** |                     |                      |        |             |
| Present/present | 66:103 | 1 \(^c\) |        |             |
| Null/present | 75:117 | 0.97 (0.63–1.50) |        |             |
| Present/null | 30:36 | 1.28 (0.71–2.30) |        |             |
| Null/null | 30:33 | 1.39 (0.77–2.53) | 0.773 | 0.109 (–0.603; 0.820) |
| **GSTM1 \(\times\) SULT1A1** |                     |                      |        |             |
| Present/present | 53:84 | 1 \(^c\) |        |             |
| Null/present | 67:96 | 1.09 (0.68–1.76) |        |             |
| Present/null | 43:55 | 1.40 (0.81–2.41) |        |             |
| Null/null | 38:54 | 1.22 (0.70–2.13) | 0.566 | nc          |
| **GSTT1 \(\times\) CYP1A1 \(^\ast\) 2A** |                     |                      |        |             |
| Present/wt homozygote | 108:169 | 1 \(^c\) |        |             |
| Null/wt homozygote | 44:56 | 1.20 (0.74–1.93) |        |             |
| Present/any mt | 33:51 | 0.98 (0.58–1.63) |        |             |
| Null/any mt | 16:13 | 2.00 (0.91–4.41) | 0.289 | 0.414 (–0.149; 0.976) |
| **GSTT1 \(\times\) SULT1A1** |                     |                      |        |             |
| Present/wt/wt | 81:138 | 1 \(^c\) |        |             |
| Null/wt | 39:42 | 1.59 (0.94–2.70) |        |             |
| Present/any mt | 60:82 | 1.40 (0.90–2.20) |        |             |
| Null/any mt | 21:27 | 1.48 (0.77–2.84) | 0.347 | nc          |
| **SULT1A1 \(\times\) CYP1A1 \(^\ast\) 2A** |                     |                      |        |             |
| wt homozygote/wt homozygote | 99:138 | 1 \(^c\) |        |             |
| mt carrier/wt homozygote | 66:88 | 1.14 (0.75–1.74) |        |             |
| wt homozygote/mt carrier | 33:62 | 1.09 (0.64–1.85) |        |             |
| mt carrier/mt carrier | 23:22 | 1.67 (0.97–3.24) | 0.493 | 0.269 (–0.330; 0.867) |

OR: odds ratio; CI: confidence interval; AP: attributable proportion; nc: not calculable.

\(^a\) Adjusted for age and sex.

\(^b\) \(P\) from test for multiplicative interaction.

\(^c\) Reference category.

wt: wild-type allele.

mt: variant-type allele.

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### 4. Discussion

Our study identified \(CYP2E1^\ast 5B\) and \(CYP2E1^\ast 6\) variant alleles associated with a reduced risk of HCC. There was a borderline increased risk for HCC among carriers of combined \(SULT1A1\) and \(CYP1A1^\ast 2A\) variant alleles. The polymorphisms in \(GSTT1\) and \(SULT1A1\) are associated with increased susceptibility to smoking-related HCC.

\(CYP2E1\) can activate N-nitrosamines and benzene contained in cigarette smoke [22] and is involved in alcohol-mediated generation of oxidative stress [23]. The expression of \(CYP2E1\) correlates with the generation of hydroxyethyl radicals and lipid peroxidation products [23]. The variant \(CYP2E1^\ast 5B\) and \(CYP2E1^\ast 6\) alleles are associated with increased transcription of \(CYP2E1\) [24] that leads to development of HCC by promoting carcinogenesis. No significant association between \(CYP2E1^\ast 6\) and HCC was reported so far [25, 26], while contradictory results were reported for the \(CYP2E1^\ast 5B\) variant allele [27–35]. A recent meta-analysis did not find \(CYP2E1^\ast 5B\) c2 allele to be associated with HCC [36], also after stratifying among Asians and white. Studies conducted among white individuals, however, were few [26, 30, 35] and included limited numbers of cases.

The favorable effect of both \(CYP2E1\) polymorphisms on HCC development is not consistent with the biological premises implying a promoting role of a high activity enzyme. However, the only study previously conducted in an Italian population [35] on \(CYP2E1^\ast 5B\) c2 allele and HCC did report a similar association, indicating a favorable role of the variant allele against HCC which deserves further investigation.

Our results suggest that up to 48% and 36% of HCC cases among smokers carrying, respectively, variant \(GSTT1\)
Table 4: Effect of the gene-smoking interaction on the development of hepatocellular carcinoma.

| Genes × smoking | Cases : controls | OR (95% CI) | P | AP (95% CI) |
|-----------------|-----------------|-------------|---|------------|
| CYP1A1<sup>+</sup> 2A × smoking<sup>c</sup> | | | | |
| wt homozygote/no | 65 : 136 | 1<sup>d</sup> | | |
| wt homozygote/yes | 98 : 90 | 2.16 (1.29–3.36) | | |
| mt carrier/no | 20 : 37 | 1.08 (0.58–2.03) | | |
| mt carrier/yes | 35 : 27 | 2.81 (1.51–5.23) | 0.680 | 0.201 (−0.328; 0.730) |
| GSTM1 × smoking<sup>c</sup> | | | | |
| Present/no | 31 : 91 | 1<sup>d</sup> | | |
| Present/yes | 62 : 48 | 4.01 (2.20–7.28) | | |
| Null/no | 48 : 81 | 1.82 (1.05–3.18) | | |
| Null/yes | 57 : 69 | 2.34 (1.32–4.23) | 0.004 | nc |
| GSTT1 × smoking<sup>c</sup> | | | | |
| Present/no | 59 : 129 | 1<sup>d</sup> | | |
| Present/yes | 81 : 91 | 1.86 (1.17–2.97) | | |
| Null/no | 20 : 43 | 0.99 (0.53–1.86) | | |
| Null/yes | 38 : 26 | 3.13 (1.69–5.82) | 0.230 | 0.480 (0.001; 0.815) |
| SULT1A1 × smoking<sup>c</sup> | | | | |
| wt homozygote/no | 52 : 103 | 1<sup>d</sup> | | |
| wt homozygote/yes | 78 : 77 | 1.93 (1.19–3.14) | | |
| mt carrier/no | 33 : 70 | 1.01 (0.59–1.75) | | |
| mt carrier/yes | 55 : 40 | 3.05 (1.73–5.40) | 0.250 | 0.363 (−0.021; 0.747) |

OR: odds ratio; CI: confidence interval; AP: attributable proportion; nc: not calculable.

<sup>a</sup> Adjusted for age and sex.

<sup>b</sup> P from test for multiplicative interaction.

<sup>c</sup> The sum does not add up to the total because of some missing values.

<sup>d</sup> Reference category.

wt: wild-type allele.

mt: variant-type allele.

and SULT1A1 alleles occurred because of gene-smoking interaction. However, we could not further stratify these two results according to quantity of smoking, as numbers were low. Smoking is recognized as a risk factor for HCC [3–5] and, together with HBV and HCV, one of the major risk factors in Europe [37], and enzymes coded by GSTT1 and SULT1A1 have their role in the metabolism of tobacco carcinogens. There is therefore a biological ground for possible synergic carcinogenic effect.

There was a borderline synergic effect of SULT1A1 and CYP1A1<sup>+</sup> 2A polymorphisms in HCC carcinogenesis. The effect of SULT1A1 polymorphism on HCC development has not been investigated so far. It has been reported, however, that an enzyme coded by SULT1A1 variant allele has twofold lower catalytic activity in detoxifying the procarcinogens [38]. The biological significance of variant CYP1A1<sup>+</sup> 2A variant allele is uncertain, but CYP1A1<sup>+</sup> 2A has been reported to increase susceptibility to several cancer types, including lung, breast, and cervical cancer [39–41]. There is therefore a rationale for a synergic effect of these two SNPs in HCC carcinogenesis.

In our study, there was no significant association between HCC and alcohol. As most of the cases have hepatitis, this could lead them to stop drinking. Consequently we were unable to perform gene-alcohol interaction analysis. Secondly, we cannot exclude a selection bias. However, since the observed frequencies of the variant alleles of CYP1A1<sup>+</sup> 2A, as well as the null genotypes of GSTM1 and GSTT1, were in line with those previously reported in the Italian population, a major impact of such bias is unlikely [35, 42]. Thirdly, our study was underpowered to perform gene-gene and gene-interaction analysis.

In conclusion, we report that CYP2E1<sup>+</sup> 5B and CYP2E1<sup>+</sup> 6 polymorphisms have a favorable effect on the development of HCC, while the polymorphisms in GSTT1 and SULT1A1 may increase HCC susceptibility among smokers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the contribution of the Italian Association for Cancer Research (AIRC; Grant number 10068). The work of Nikola Panic was supported by the ERAWEB project, funded with support of the European Community. The work of Federica Turati was supported by a fellowship from the Italian Foundation for Cancer Research.
References

[1] International Agency on Research of Cancer, 2012, http://globocan.iarc.fr/Default.aspx.

[2] M. op den Winkel, D. Nagel, J. Sappel et al., “Prognosis of patients with hepatocellular carcinoma: validation and ranking of established staging-systems in a large Western HCC-Cohort,” PLoS ONE, vol. 7, no. 10, Article ID e45066, 2012.

[3] S. Chuang, C. L. Vecchia, and P. Boffetta, “Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection,” Cancer Letters, vol. 286, no. 1, pp. 9–14, 2009.

[4] S. Franceschi, M. Montella, J. Polesel et al., “Hepatitis viruses, alcohol, and tobacco in the etiology of hepatocellular carcinoma in Italy,” Cancer Epidemiology Biomarkers and Prevention, vol. 15, no. 4, pp. 683–689, 2006.

[5] F. Turati, R. Talamini, C. Pelucchi et al., “Metabolic syndrome and hepatocellular carcinoma risk,” British Journal of Cancer, vol. 108, no. 1, pp. 222–228, 2013.

[6] F. Bravi, C. Bosetti, A. Tavani, S. Gallus, and C. La Vecchia, “Coffee reduces risk for hepatocellular carcinoma: an updated meta-analysis,” Clinical Gastroenterology and Hepatology, vol. 11, no. 11, pp. 1413–1421, 2013.

[7] Y. Gao, Q. Jiang, X. Zhou et al., “HBV infection and familial aggregation of liver cancer: an analysis of case-control family study,” Cancer Causes and Control, vol. 15, no. 8, pp. 845–850, 2004.

[8] F. Jin, W. J. Xiong, J. C. Jing, Z. Feng, L. S. Qu, and X. Z. Shen, “Evaluation of the association studies of single nucleotide polymorphisms and hepatocellular carcinoma: a systematic review,” Journal of Cancer Research and Clinical Oncology, vol. 137, no. 7, pp. 1095–1104, 2011.

[9] J. A. Hasler, “Pharmacogenetics of cytochromes P450,” Molecular Aspects of Medicine, vol. 20, no. 1-2, pp. 12–137, 1999.

[10] J. D. Hayes and D. J. Pulford, “The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance,” Critical Reviews in Biochemistry and Molecular Biology, vol. 30, no. 6, pp. 445–600, 1995.

[11] N. Gamage, A. Barnett, N. Hempel et al., “Human sulfotransferases and their role in chemical metabolism,” Toxicological Sciences, vol. 90, no. 1, pp. 5–22, 2006.

[12] K. Song, J. Yi, X. Shen, and Y. Cai, “Genetic polymorphisms of glutathione S-transferase genes GSTM1, GSTT1 and risk of hepatocellular carcinoma,” PLoS ONE, vol. 7, no. 11, Article ID e48924, 2012.

[13] L. Yu, L. Sun, Y. Jiang, B. Lu, D. Sun, and L. Zhu, “Interactions between CYP1A1 polymorphisms and cigarette smoking are associated with the risk of hepatocellular carcinoma: evidence from epidemiological studies,” Molecular Biology Reports, vol. 39, no. 6, pp. 6641–6646, 2012.

[14] J. Bruix and M. Sherman, “Management of Hepatocellular carcinoma,” Hepatology, vol. 42, no. 5, pp. 1208–1236, 2005.

[15] M. Arand, R. Mühlbauer, J. Hengstler et al., “A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase GSTM1 and GSTT1 polymorphisms,” Analytical Biochemistry, vol. 236, no. 1, pp. 184–186, 1996.

[16] M. W. Coughtrie, R. A. Gilissen, B. Shek et al., “Phenol sulphotransferase SULT1A1 polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations,” Biochemical Journal, vol. 337, no. 1, pp. 45–49, 1999.

[17] N. E. Breslow and N. E. Day, Statistical Methods in Cancer Research, Vol. 1. The Analysis of Case-Control Studies, IARC, Lyon, France, 1980.

[18] K. F. Hirji, C. R. Mehta, and N. R. Patel, “Exact inference for matched case-control studies,” Biometrics. Journal of the Biometric Society, vol. 44, no. 3, pp. 803–814, 1988.

[19] K. J. Rothman, “Measuring interactions,” in Epidemiology: An Introduction, K. J. Rothman, Ed., pp. 168–190, Oxford University Press, New York, NY, USA, 2002.

[20] T. Andersson, L. Alfredsson, H. Kullberg, S. Zdravkovic, and A. Ahlborn, “Calculating measures of biological interaction,” European Journal of Epidemiology, vol. 20, no. 7, pp. 575–579, 2005.

[21] J. Little, J. P. T. Higgins, J. P. A. Ioannidis et al., “Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE statement,” Italian Journal of Public Health, vol. 6, no. 3, pp. 238–255, 2009.

[22] F. P. Guengerich, D.-H. Kim, and M. Iwasaki, “Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects,” Chemical Research in Toxicology, vol. 4, no. 2, pp. 168–179, 1991.

[23] E. Albano, P. Clot, M. Morimoto, A. Tomasi, M. Ingelman-Sundberg, and S. W. French, “Role of cytochrome P4502E1-dependent formation of hydroxysterol free radical in the development of liver damage in rats intragastrically fed with ethanol,” Hepatology, vol. 23, no. 1, pp. 155–163, 1996.

[24] S.-M. Wang, A.-P. Zhu, D. Li, Z. Wang, P. Zhang, and G. Zhang, “Frequencies of genotypes and alleles of the functional SNPs in CYP2C19 and CYP2E1 in mainland chinese kazakh, uygur and han populations,” Journal of Human Genetics, vol. 54, no. 6, pp. 372–375, 2009.

[25] M.-W. Yu, A. Gladek-Yarborough, S. Chimpraseret al., “Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma,” Gastroenterology, vol. 109, no. 4, pp. 1266–1273, 1995.

[26] N. A. Wong, F. Rae, K. J. Simpson, G. D. Murray, and D. J. Harrison, “Genetic polymorphisms of cytochrome p4502E1 and susceptibility to alcoholic liver disease and hepatocellular carcinoma in a white population: a study and literature review, including meta-analysis,” Journal of Clinical Pathology—Molecular Pathology, vol. 53, no. 2, pp. 88–93, 2000.

[27] M. Munaka, K. Kohshi, T. Kawamoto et al., “Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma,” Journal of Cancer Research and Clinical Oncology, vol. 129, no. 6, pp. 355–360, 2003.

[28] T. Koida, T. Ohno, X. E. Huang et al., “HBV/HCV infection, alcohol, tobacco and genetic polymorphisms for hepatocellular carcinoma in Nagoya, Japan,” Asian Pacific Journal of Cancer Prevention, vol. 1, no. 3, pp. 237–243, 2000.

[29] X. P. Ye, T. Peng, T. W. Liu et al., “The effect of interaction between alcohol drinking and polymorphisms of cytochrome P450 2E1 on the susceptibility of Hepatocellular carcinoma in Guangxi region,” Journal of Guangxi Medical University, vol. 25, no. 4, pp. 493–495, 2008 (Chinese).
[30] J. M. Ladero, J. A. Agúndez, A. Rodriguez-Lescure, M. Diaz-Rubio, and J. Benítez, “RsaI polymorphism at the cytochrome P4502E1 locus and risk of hepatocellular carcinoma,” *Gut*, vol. 39, no. 2, pp. 330–333, 1996.

[31] T. Imaizumi, Y. Higaki, M. Hara et al., “Interaction between cytochrome P450 1A2 genetic polymorphism and cigarette smoking on the risk of hepatocellular carcinoma in a Japanese population,” *Carcinogenesis*, vol. 30, no. 10, pp. 1729–1734, 2009.

[32] N. A. Wong, F. Rae, K. J. Simpson, G. D. Murray, and D. J. Harrison, “Genetic polymorphisms of cytochrome p4502E1 and susceptibility to alcoholic liver disease and hepatocellular carcinoma in a white population: a study and literature review, including meta-analysis,” *Molecular Pathology*, vol. 53, no. 2, pp. 88–93, 2000.

[33] S. Z. Yu, X. E. Huang, T. Koide et al., “Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China,” *Japanese Journal of Cancer Research*, vol. 93, no. 12, pp. 1287–1292, 2002.

[34] H. S. Lee, J. H. Yoon, S. Kamimura et al., “Lack of association of cytochrome P450 2E1 genetic polymorphisms with the risk of human hepatocellular carcinoma,” *International Journal of Cancer*, vol. 71, no. 5, pp. 737–740, 1997.

[35] L. Silvestri, L. Sonzogni, A. De Silvestri et al., “CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer,” *International Journal of Cancer*, vol. 104, no. 3, pp. 310–317, 2003.

[36] C. Liu, H. Wang, C. Pan, J. Shen, and Y. Liang, “CYP2E1 PstI/RsaI polymorphism and interaction with alcohol consumption in hepatocellular carcinoma susceptibility: evidence from 1,661 cases and 2,317 controls,” *Tumour Biology*, vol. 33, no. 4, pp. 979–984, 2012.

[37] D. Trichopoulos, C. Bamia, P. Lagiou et al., “Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study,” *Journal of the National Cancer Institute*, vol. 103, no. 22, pp. 1686–1695, 2011.

[38] S. Nowell, C. B. Ambrosone, S. Ozawa et al., “Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfotransferase phenotype in platelet cytosol,” *Pharmacogenetics*, vol. 10, no. 9, pp. 789–797, 2000.

[39] T. Juárez-Cedillo, M. Vallejo, J. M. Fragoso et al., “The risk of developing cervical cancer in Mexican women is associated to CYPIA1 MspI polymorphism,” *European Journal of Cancer*, vol. 43, no. 10, pp. 1590–1595, 2007.

[40] A. Shin, D. Kang, J. Y. Choi et al., “Cytochrome P450 1A1 (CYPIA1) polymorphisms and breast cancer risk in Korean women,” *Experimental and Molecular Medicine*, vol. 39, no. 3, pp. 361–366, 2007.

[41] P. Zhan, Q. Wang, Q. Qian, S. Wei, and L. Yu, “CYP1A1 MspI and exon7 gene polymorphisms and lung cancer risk: an updated meta-analysis and review,” *Journal of Experimental and Clinical Cancer Research*, vol. 30, no. 1, article 99, 2011.

[42] L. Covolo, U. Gelatti, R. Talamini et al., “Alcohol dehydrogenase 3, glutathione S-transferase M1 and T1 polymorphisms, alcohol consumption and hepatocellular carcinoma (Italy),” *Cancer Causes and Control*, vol. 16, no. 7, pp. 831–838, 2005.
