Null Results in Brief

Lack of Association between the Functional Polymorphisms in the Estrogen-Metabolizing Genes and Risk for Hepatocellular Carcinoma

Xiaoyan Yuan,[1,2] Gangqiao Zhou,[1,2] Yun Zhai,[1,2] Weimin Xie,[3] Ying Cui,[3] Jia Cao,[1,2] Lianteng Zhi,[1,2] Hongxing Zhang,[1,2] Hao Yang,[1,2] Xiaoai Zhang,[1,2] Wei Qiu,[1,2] Yong Peng,[3] Xiumei Zhang,[1,2] Ling Yu,[1,2] Xia Xia,[1,2] and Fuchu He[1,2,4]

[1]The State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing, China; [2]Department of Hygienic Toxicology, Preventive Medical College, Third Military Medical University, Chongqing, China; [3]Cancer Institute of Guangxi, Nanning, Guangxi, China; and [4]Institute of Biomedical Sciences, Fudan University, Shanghai, China

Abstract

Estrogens have been proposed to act as tumor promoters and induce hepatocarcinogenesis. Recently, we observed a significant association between the risk for hepatocellular carcinoma and the polymorphisms of the estrogen receptor (ESR) α (*ESR1*) gene, supporting the hypothesis of involvement for the estrogen-ESR axis in the estrogen-induced hepatocarcinogenesis. In this study, based on another hypothesis in which estrogen metabolites can directly cause DNA damage and affect tumor initiation, we examined whether the polymorphisms of the estrogen-metabolizing enzymes (EME), which are involved in biogenesis (CYP17, CYP19), bioavailability (CYP1A1, CYP1B1), and degradation (catechol-*O*-methyltransferase) of the estrogens, have any bearing on the risk for hepatocellular carcinoma. Seven functional polymorphisms in five *EMEs* (*CYP17 Msp*AI site, *CYP19* Trp39Arg, Ile462Val and *Msp*I site in *CYP1A1*, *CYP1B1* Val432Leu, and Ala72Ser and Val158Met in *catechol-O-methyltransferase*) were genotyped in 434 patients with hepatocellular carcinoma and 480 controls by PCR-RFLP analysis. The associations between the polymorphisms and hepatocellular carcinoma risk were evaluated while controlling for confounding factors. No significant association with the risk for hepatocellular carcinoma was observed with the seven polymorphisms in hepatitis B virus carriers and non–hepatitis B virus carriers after correction for multiple comparisons. After stratification by common confounding factors of hepatocellular carcinoma, the *EME* polymorphism remained no significant association with the hepatocellular carcinoma risk. Furthermore, no signs of gene-gene interactions were observed for each combination of the seven polymorphisms. Our findings suggest that the polymorphisms of *EMEs* may not contribute significantly to the risk for hepatocellular carcinoma. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3621–7)

Introduction

Animal models and human epidemiologic studies have suggested that estrogens act as tumor promoters and might induce hepatocarcinogenesis (1-4). The estrogens exert the effects by binding to their receptors [estrogen receptors (ESR)]. In a recent study, we have hypothesized that the genetic polymorphisms within *ESRs* could influence the effects of estrogens, which in turn results in genotype-dependent differences in risk for hepatocellular carcinoma. Indeed, the polymorphisms in the 5′ end of the ESR α (*ESR1*) gene have been shown to be associated with an increased hepatocellular carcinoma risk, supporting the hypothesis of involvement for the estrogen-ESR axis in the estrogen-induced hepatocarcinogenesis (5).

In the present study, we focused on another hypothesis. Several studies have shown that estrogen metabolites can bind to DNA and trigger damage, suggesting that the estrogens might be endogenous genotoxic agents that can directly cause genetic alteration and affect tumor initiation (6, 7). This possibility is supported by the finding that women with reduced amounts of the enzymes responsible for removing reactive estrogen metabolites are at higher risk of developing breast cancer (8). Based on the obvious relevance of estrogens in human hepatocarcinogenesis (3), it is reasonable to anticipate that the same events involved in hepatocellular carcinoma. We therefore hypothesize that the estrogen-metabolizing enzymes (EME), which are involved in the biogenesis, bioavailability, and degradation of estrogens, may also be the excellent biological candidate susceptibility genes for hepatocellular carcinoma. It is expected that the polymorphisms within the *EMEs* may

Received 8/10/08; revised 9/19/08; accepted 9/29/08.

Grant support: Chinese National Science Fund for Creative Research Groups Program grant 30621063 (F. He) and Chinese High-tech Program grant 2006AA02A412, Chinese National Basic Research Program grant 2006CB910803, Beijing Science & Technology NOVA Program grant 2006A54, and Chinese Key Project for the Infectious Diseases 2008ZX10002-016 (G. Zhou).

Requests for reprints: Gangqiao Zhou and Fuchu He, The State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing 100850, China. Phone/Fax: 86-10-80705255 or 86-10-68177417. E-mail: zhougq@chgb.org.cn and hefc@nic.bmi.ac.cn

doi:10.1158/1055-9965.EPI-08-0742

Table 1. Primers and restriction enzymes used in *EME* polymorphisms genotyping by PCR-RFLP analysis

Polymorphisms	Primers sequences*	Amplicon size (bp)	Restriction enzymes	Digest size (bp)
*CYP17 Msp*AI site	5′-TGGCTGGGTGCCG GCAGGCAAGATAGAC*c*GC-3′ (forward) 5′-TCCCTTACCTAGCTCCTCCTC-3′ (reverse)	261	*ACC* I I	A1/A1: 261 A2/A2: 231, 30 A1/A2: 261, 231, 30
CYP19 Trp39Arg	5′-ATGACTGACTTACCTGGTATTGAGGATGTGCCCTCATAATT*g*C-3′ (forward) 5′-CCTCTGAAGCAACAGGAGCTA-3′ (reverse)	271	*Hin*6I	Trp/Trp: 271 Arg/Arg: 228, 43 Trp/Arg: 271, 228, 43
CYP1A1 Ile462Val	5′-GATAGCCAGGAAGAGAAAGACCTCCCAGCGG*tt*AA-3′ (forward) 5′-TCCCTCTGGTTACAGGAAGCTA-3′ (reverse)	183	*Ksp* AI	Ile/Ile: 183 Val/Val: 150,33 Ile/Val: 183, 150,33
*CYP1A1 Msp*I site	5′-CAGTGAAGAGGTGTAGCCGCT-3′ (forward) 5′-TAGGAGTCTTGTCTCATGCCT-3′ (reverse)	342	*Msp*I	wt/wt: 342 m1/m1: 207, 135 wt/m1: 342, 207, 135
CYP1B1 Val432Leu	5′-TTGGCCCTGAAATCGCAC*c*GGT-3′ (forward) 5′-CCAAGGACACTGTGGTTTTTGTCAA*g*CAG-3′ (reverse)	240	*Bse*NI	Leu/Leu: 240 Val/Val: 194, 46 Leu/Val: 240, 194, 46
COMT Ala72Ser	5′-TGCTGTTGGCAGCTGTGT-3′ (forward) 5′-GTAGGTGTCAATGGCCTCCAGCACGCTCTG*c*G-3′ (reverse)	239	*ACC* I I	Ser/Ser: 239 Ala/Ala: 208, 31 Ser/Ala: 239, 208, 31
COMT Val158Met	5′-CTCTCCTCCGTCCCCAAC-3′ (forward) 5′-AACGGGTCAGG*a*ATGCACACCTTGTCCT*g*CA-3′ (reverse)	241	*Sph*I	Val/Val: 241 Met/Met: 212, 29 Val/Met: 241, 212, 29

*Italicized lowercase letters are the base mismatches.

Table 2. The genotype and allele frequencies of 7 *EME* polymorphisms in patients with hepatocellular carcinoma and controls

Polymorphisms	HBV carriers			Non–HBV carriers		
	Cases/controls	OR (95% CI)*	*P**	Cases/controls	OR (95% CI)*	*P**
*CYP17 Msp*AI site						
A2/A2	113/70	Reference		37/124	Reference	
A2/A1	148/84	1.07 (0.71-1.61)	0.75	54/132	1.38 (0.84-2.28)	0.20
A1/A1	61/30	1.24 (0.72-2.12)	0.44	14/38	1.34 (0.64-2.84)	0.44
A1 allele	0.42/0.39			0.39/0.35		
CYP19 Trp39Arg						
Trp/Trp	297/173	Reference		98/277	Reference	
Trp/Arg	16/13	1.32 (0.61-2.85)	0.48	4/17	1.55 (0.48-4.94)	0.46
Arg/Arg	0/0	NA	NA	1/0	NA	NA
Arg allele	0.026/0.035			0.029/0.029		
CYP1A1 Ile462Val						
Ile/Ile	157/97	Reference		56/126	Reference	
Ile/Val	118/70	1.03 (0.69-1.53)	0.86	37/137	0.62 (0.37-1.01)	0.057
Val/Val	28/17	1.04 (0.53-2.05)	0.90	5/30	0.40 (0.14-1.14)	0.086
Val allele	0.29/0.28			0.24/0.34		
*CYP1A1 Msp*I site						
m1/m1	75/50	Reference		21/88	Reference	
m1/wt	145/86	1.13 (0.71-1.79)	0.61	50/134	1.46 (0.80-2.66)	0.22
wt/wt	76/49	1.14 (0.68-1.93)	0.62	25/72	1.45 (0.73-2.86)	0.28
wt Allele	0.50/0.50			0.52/0.47		
CYP1B1 Val432Leu						
Leu/Leu	290/168	Reference		97/273	Reference	
Leu/Val	32/18	1.06 (0.57-1.97)	0.85	8/21	1.12 (0.46-2.70)	0.81
Val/Val	2/0	NA	NA	0/0	NA	NA
Val allele	0.056/0.048			0.038/0.036		
COMT Ala72Ser						
Ala/Ala	298/180	Reference		100/283	Reference	
Ala/Ser	24/4	4.06 (1.37-12.05)	0.012	5/9	1.63 (0.52-5.16)	0.41
Ser/Ser	0/0	NA	NA	1/1	3.15 (0.19-52.07)	0.42
Ser allele	0.037/0.011			0.033/0.019		
COMT Val158Met						
Val/Val	204/113	Reference		54/173	Reference	
Val/Met	101/60	0.91 (0.61-1.36)	0.65	43/97	1.46 (0.89-2.39)	0.14
Met/Met	12/10	0.73 (0.30-1.75)	0.48	6/22	0.81 (0.30-2.23)	0.69
Met allele	0.20/0.22			0.27/0.24		

NOTE: The frequencies of genotypes are indicated in absolute values. The number of samples genotyped varies because of genotyping failure for some individuals. The HBV carriers are subjects positive for hepatitis B surface antigen and anti–hepatitis B core antigen for at least 6 mo. All odds ratios and *P*s are adjusted for age, gender, status of smoking and drinking, pack-years of smoking, and family history of hepatocellular carcinoma.
Abbreviations: OR, odds ratio; NA, not applicable.
*No correction was made for testing multiple polymorphisms.

contribute to interindividual differences of levels of estrogen metabolites and then potentially affect the risk for hepatocellular carcinoma.

Several functional polymorphisms of the *EMEs*, which are thought to affect the respective EME activity in an allele-specific manner, have been well characterized. Cytochrome *P*450c17α (CYP17) is a key enzyme in estrogen biosynthesis. In the 5′-untranslated region of *CYP17*, a T→C transition 34 bp upstream of the translation initiation site generates an *Msp*AI restriction site (known as *A2* allele). This polymorphism creates a new Sp1-type (CCACC box) binding site, providing an additional promoter activity and thus increasing the estrogen biosynthesis (9, 10). CYP19 is another key enzyme in estrogen biosynthesis. A nonsynonymous polymorphism, Trp39Arg, which is caused by a substitution of arginine for tryptophan at codon 39 of *CYP19*, results in the lack of estrogen biosynthesis *in vitro* (11).

Two other CYP enzymes, CYP1A1 and CYP1B1, are involved in the hydroxylation of estrogen. To date, two functional polymorphisms have been identified. One is the nonsynonymous polymorphism Ile462Val at codon 462 in the heme-binding region; the other is a T→C transition at 3′-noncoding region, which creates an *Msp*I restriction site (known as *m1* allele). Both of the polymorphisms are found to contribute to elevated enzyme activity (12, 13). In the *CYP1B1* gene, a nonsynonymous polymorphism, Val432Leu, which is caused by a substitution of valine to leucine at codon 432 in exon 3, is linked to a higher catalytic activity (14).

Catechol-*O*-methyltransferase (COMT) is among the major enzymes responsible for inactivation of catechol estrogens, which are major metabolites of estrogens and can cause oxidative DNA damage. It has been shown that two nonsynonymous polymorphisms, Ala72Ser at codon 72 in exon 3 and Val158Met at codon 158 in exon 4, cause a dramatic reduction in enzyme activity (15, 16).

Several studies have shown these functional polymorphisms to be associated with a wide spectrum of cancers, including breast (17, 18), endometrial (19), ovarian (20), and prostate (21). In the present study, we examined whether the polymorphisms of

Table 3. Haplotype distribution of *CYP1A1* and *COMT* in patients with hepatocellular carcinoma and controls

Haplotypes	HBV carriers			Non–HBV carriers		
	Cases/controls	OR (95% CI)*	*P**	Cases/controls	OR (95% CI)*	*P**
CYP1A1						
Ile-wt	272/179	Reference		99/268	Reference	
Val-m1	146/101	0.96 (0.70-1.30)	0.39	44/187	0.63 (0.42-0.94)	0.019
Ile-m1	146/85	1.20 (0.86-1.70)	0.56	46/121	1.03 (0.68-1.54)	0.31
Val-wt	24/3	4.41 (1.34-14.60)	0.0096	1/10	0.27 (0.034-2.23)	0.21
COMT						
Ala-Val	501/286	Reference		150/436	Reference	
Ala-Met	125/80	0.92 (0.67-1.30)	0.39	55/141	1.20 (0.81-1.70)	0.58
Ser-Val	24/4	3.62 (1.23-10.60)	0.013	7/11	1.70 (0.71-4.20)	0.27

NOTE: The sums of haplotypes vary because of genotyping failure for some individuals. All odds ratios and *P*s are adjusted for age, gender, status of smoking and drinking, pack-years of smoking, and family history of hepatocellular carcinoma.
*No correction was made for testing multiple polymorphisms.

EMEs have any bearing on the risk for hepatocellular carcinoma in hepatitis B virus (HBV) carriers and non–HBV carriers.

Materials and Methods

Patients and Controls. This case-control study consists of 434 incident patients with hepatocellular carcinoma and 480 control subjects. The diagnosis of cases, the inclusion and exclusion criteria for cases and controls, and the definition of HBV carriers, smokers, and drinkers were described in detail in our previous studies (5, 22). Briefly, the diagnosis of hepatocellular carcinoma was made by either positive histologic findings or an elevated α-fetoprotein level (≥400 ng/mL) combined with at least one positive image on the angiography, sonography, and/or high-resolution contrast computerized tomography. The controls had no evidence of hepatocellular carcinoma based on ultrasonography and serum α-fetoprotein level, and no individual history of other cancers. All participants were negative for antibodies to hepatitis C virus, hepatitis D virus, or HIV and had no any other type of liver disease such as autoimmune hepatitis, toxic hepatitis, and primary biliary cirrhosis or Budd-Chiari syndrome. At recruitment, informed consent was obtained from each subject, and personal information on demographic factors, medical history, history of cigarette smoking and alcohol drinking, and family history of cancer were collected via structured questionnaire. This study was done with the approval of the Medical Ethical Committee of Chinese National Human Genome Center.

Genotyping of Polymorphisms. Seven polymorphisms, including *CYP17 Msp*AI site (rs743572), *CYP19* Trp39Arg (rs2236722), *CYP1A1* Ile462Val (rs1048943), *Msp*I site (rs4646903), *CYP1B1* Val432Leu (rs1056836), *COMT* Ala72Ser (rs6267), and Val158Met (rs4680), were genotyped by PCR-RFLP analysis. Briefly, the DNA sequence containing the relevant polymorphic site was amplified by PCR, and then the amplicon was digested with an appropriate restriction enzyme that cleaves only one of the two alleles. The digests were then subjected to gel electrophoresis and visualized by ethidium bromide staining. The PCR primers used in the aforementioned PCR-RFLP assays and the appropriate restriction enzymes are described in Table 1.

Genotyping was done by staff blinded to the subjects' case or control status. The accuracy of genotyping data for polymorphisms obtained from PCR-RFLP analyses was tested by direct DNA sequencing of a 15% masked, random sample of cases and controls, and all results were in 100% concordance.

Statistical Analyses. Genotype and allele frequencies for each polymorphism were determined by direct gene counting. The fitness to Hardy-Weinberg equilibrium was tested using the random-permutation procedure implemented in the Arlequin package (available at http://lgb.unige.ch/arlequin/). The associations between the polymorphisms and risk for hepatocellular carcinoma were evaluated by multiple logistic regression analyses while controlling for confounding factors (including age, sex, status of smoking and drinking, pack-years of smoking, and family history), and the *P*s, odds ratios, and 95% confidence intervals (95% CI) were calculated. Potential modification of the effect of the polymorphisms on hepatocellular carcinoma risk was assessed for the above confounding factors by the addition of interaction terms in the logistic model and by stratification analyses of subgroups of subjects determined by these factors. In view of the multiple testing, the correction factor $n \times (m - 1)$, where n means loci with m alleles each, was applied to correct the significance level. $P < 0.0071$ (= 0.05 / 7) was considered to be statistically significant, and all statistical tests were two sided. These analyses were done using SPSS software (version 9.0, SPSS, Inc.).

Haplotypes based on the polymorphisms Ile462Val and *Msp*I in *CYP1A1* and Ala72Ser and Val158Met in *COMT* were inferred using program PHASE 2.1 (available at http://www.stat.washington.edu/stephens/). The pairwise linkage disequilibrium index (Lewontin's D' and r^2) calculation was done using the Arlequin package. Haplotype frequencies between cases and controls were compared using χ^2 test; haplo.glm program (available at http://rss.acs.unt.edu/Rdoc/library/haplo.stats/html/haplo.glm.html) was then done to calculate adjusted odds ratios and *P*s while adjusting for age, gender, status of smoking and drinking, pack-years of

smoking, and family history of hepatocellular carcinoma for each haplotype, and the number of simulations for empirical *P*s was set as 1,000. For high-order gene-gene interactions, multifactor dimensionality reduction analysis was done using multifactor dimensionality reduction software (version 1.0.0, available at http:/www.epistasis.org/mdr.html). Multifactor dimensionality reduction is a nonparametric and genetic model–free approach and can be used in case-control and discordant sib-pair study designs (23). Cross-validation consistency and balanced accuracy estimates were calculated for each combination of a pool of polymorphisms. The model with the highest accuracy and maximal cross-validation was considered to be the best. The statistical significance was determined by comparing the accuracy of the observed data with the distribution of accuracy under the null hypothesis of no associations derived empirically from 1,000 replicates of permutations.

Results

The genotyping results of seven polymorphisms are presented in Table 2. The genotype distribution of the *COMT* Ala72Ser polymorphism conformed to the Hardy-Weinberg equilibrium ($P > 0.05$) in patients but not in controls ($P = 0.009$). However, all the other six polymorphisms conformed to the Hardy-Weinberg equilibrium in patients and controls ($P > 0.05$). On the basis of logistic regression analysis with adjustment for age, sex, status of smoking and drinking, and family history, a significant association with the risk for hepatocellular carcinoma was observed for the *COMT* Ala72Ser in HBV carriers (Table 2). An increased risk for hepatocellular carcinoma was found to be associated with the *Ala/Ser* genotype, with the odds ratio being 4.06 (95% CI = 1.37-12.05; $P = 0.012$) compared with the *Ala/Ala* genotype. However, after correction for multiple comparisons, the association was never again significant. For the other six polymorphisms, that is, *CYP17 Msp*AI site, *CYP19* Trp39Arg, *CYP1A1* Ile462Val and *Msp*I site, *CYP1B1* Val432Leu, and *COMT* Val158Met, we found no association with the risk for hepatocellular carcinoma in HBV carriers and non–HBV carriers (Table 2). The associations between these polymorphisms and the risk for hepatocellular carcinoma were further examined with stratification by age, sex, family history, status of smoking and drinking, and pack-years of smoking. Again, no significant association was found in HBV carriers and non–HBV carriers while correcting for multiple testing (data not shown).

Furthermore, we did the haplotype analysis for evaluating the haplotype frequencies of polymorphisms located nearby at the same gene regions, trying to derive haplotypes specifically correlated with hepatocellular carcinoma. The linkage disequilibrium analyses showed that the Ile462Val and *Msp*I in *CYP1A1* and the Ala72Ser and Val158Met in *COMT* are in strong linkage disequilibrium (for *CYP1A1*: $|D'| = 0.85$, $r^2 = 0.30$, $P < 0.001$; for *COMT*: $|D'| = 1.00$, $r^2 = 0.30$, $P < 0.001$). Haplotypes based on the polymorphisms Ile462Val and *Msp*I in *CYP1A1* and Ala72Ser and Val158Met in *COMT* were then constructed, respectively. Four haplotypes of *CYP1A1* were observed, and only 3 haplotypes had allele frequency of >5%. For *COMT*, three haplotypes were observed, and only two haplotypes had allele frequency of >5%. The estimated haplotype distribution in these two genes was not significantly different between the patients with hepatocellular carcinoma and controls in HBV carriers and non–HBV carriers after correction for multiple comparisons (Table 3).

Epistasis or gene-gene interaction is increasingly assumed to play a crucial role in the genotype-to-phenotype relationship of common diseases. Thus, a nonparametric and genetic model–free approach, multifactor dimensionality reduction analysis, was done to explore the potential gene-gene interactions. However, we did not detect any statistically significant interactive effect for each combination of seven polymorphisms in our case-control data set (data not shown).

Discussion

In the present study, we assessed whether there was an association between the *EME* functional polymorphisms and the risk for HBV-related and non-HBV-related hepatocellular carcinoma. However, no significant association was observed in our case-control population. In addition, no evidence for gene-gene interactions was observed. It would be expected that the effects of *EME* genotypes may be masked and only become detectable in the presence of certain conditions. Indeed, many factors such as chronic infection with HBV, male gender, family history, smoking, and alcoholic consumption have been shown as independent risk factors for hepatocellular carcinoma (24-26). However, we did not find a statistically significant interaction between the *EME* polymorphisms and these risk factors, suggesting that these factors may not have modification effect on the susceptibility to hepatocellular carcinoma related to *EME* genotypes. These results thus do not support the hypothesis that the *EME* polymorphisms might modify susceptibility to hepatocellular carcinoma.

Previous study suggested that the endogenous hormonal environment in humans might modify the association between the high-risk *EME* genotypes and increased breast cancer risk; the cancer risk related to the *EME* genotypes was stronger in women with prolonged estrogen exposure and higher estrogen levels compared with that with a shorter duration of estrogen exposure and lower estrogen levels (27). On the basis of the obvious relevance of endogenous estrogens in human hepatocarcinogenesis, it is reasonable to anticipate the same events involved in hepatocellular carcinoma. Unfortunately, the information on personal estrogen exposure was not obtained in the present study. Additional studies investigating the interaction between the estrogen exposure and *EME* polymorphisms in hepatocellular carcinoma should be required in the future.

The genotype distribution of *COMT* Ala72Ser deviated from Hardy-Weinberg proportions in controls ($P = 0.009$); however, the concordance rate for the quality control samples ($n = 152$), which were randomly selected from cases and controls, was 100% for all the seven polymorphisms, including the *COMT* Ala72Ser. Therefore, we do not believe that the deviation from Hardy-Weinberg equilibrium for this polymorphism is due to genotyping error.

Evidences supporting the roles for polymorphisms in interindividual variation of EME activity *ex vivo* have been extensively analyzed in several studies (9-16). Moreover, several lines of evidence indicate that the *EME* polymorphisms can influence individual susceptibility to the development of hormone-related cancers (17-21). In view of the biological role for EMEs in metabolism of estrogens and the obvious biological plausibility of estrogen metabolites in hepatocarcinogenesis (3), the following factors may have contributed to lack of an association between the *EME* polymorphisms and hepatocellular carcinoma. First, additional polymorphisms that alter EME activity are likely. Our polymorphism selection strategy focused on variants with functional significance; we did not fully characterize risk in relation to all polymorphisms in these genes. Thus, variation in EME activity in human hepatocellular carcinoma may be only partly explained by these seven functional polymorphisms. Second, inadequate power may be an explanation for our negative results. This study had >85% power at a significance of 0.05 to detect a recessive allele with a minor allele frequency of 0.20 that confers a risk of 1.4. Thus, additional larger population-based case-control studies are warranted. Lastly, our negative results may be due to inherent selection bias. As a hospital-based study, our hepatocellular carcinoma cases were enrolled from the hospitals and the control subjects were selected from the community population, inherent selection bias cannot be completely excluded. However, by matching age and residential area, relying on covariate adjustments in the final analysis, and using analyses stratified by potential confounders, the potential selection bias might have been minimized.

The *EME* polymorphisms and hepatocellular carcinoma risk have been investigated previously, but the results are conflicting. Yin et al. (28) have reported an association of the high-activity *m1* allele of *CYP1A1 Msp*I site and the increased risk for hepatocellular carcinoma in Taiwan females. Furthermore, the women harboring two or three high-risk genotypes (including the high-activity *CYP17 A2*, high-activity *CYP1A1 m1*, and low-activity *COMT 158Met* alleles) had significantly increased risk for hepatocellular carcinoma compared with those harboring no or one variant. In contrast, in agreement with our present results, some reports also showed no association between certain *EME* polymorphism and risk for hepatocellular carcinoma. For instance, no association of the two *CYP1A1* polymorphisms, *Msp*I and Ile462Val, was observed with the hepatocellular carcinoma risk in either chronic hepatitis B carriers or hepatitis C virus–infected patients (29, 30). It was also reported that the *CYP17 Msp*AI and *COMT* Val158Met are not associated with the risk for hepatitis C virus–related hepatocellular carcinoma (31). The conflicting results could be attributable to the differences in demography, ethnicity, lifestyles, type of viral infections, and clinical settings. In addition, other methodologic factors in the studies, such as small sample size, inadequate adjustment for confounding factors, or lack of correction for multiple testing, could also cause the inconsistent results.

In summary, our findings do not support associations between the seven functional polymorphisms of *EME* genes and the risk for hepatocellular carcinoma, suggesting that these *EME* polymorphisms might not involved in the predisposition to develop hepatocellular carcinoma. However, additional studies are warranted before the importance of *EME* polymorphisms in the etiology of hepatocellular carcinoma can be fully ascertained. First, data from larger population-based case-control studies among Chinese and from ethnically diverse populations are required to confirm our observation. Second, the other potentially functional polymorphisms in these *EME* genes and their associations with hepatocellular carcinoma risk should be systematically investigated. Lastly, investigation of additional polymorphic genes participating in the estrogen-metabolizing pathways, for example, steroid sulfatase, estrogen sulfotransferase, and 17β-hydroxysteroid dehydrogenase, should also be of interest.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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

We thank all the tested individuals, their families, and collaborating clinicians for their participation.

References

1. Nelson R. Steroidal oestrogens added to list of known human carcinogens. Lancet 2002;360:2053.
2. Coe JE, Ishak KG, Ross MJ. Estrogen induction of hepatocellular carcinomas in Armenian hamsters. Hepatology 1990;11:570–7.
3. Neuberger J, Forman D, Doll R, Williams R. Oral contraceptives and hepatocellular carcinoma. Br Med J 1986;292:1355–7.
4. Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004;127:S72–8.
5. Zhai Y, Zhou G, Deng G, et al. Estrogen receptor α polymorphisms associated with susceptibility to hepatocellular carcinoma in hepatitis B virus carriers. Gastroenterology 2006;130:2001–9.
6. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents—DNA adducts and mutations. J Natl Cancer Inst Monogr 2000;27:75–93.
7. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA. Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. Carcinogenesis 2000;21:1355–63.
8. Lavigne JA, Helzlsouer KJ, Huang HY, et al. An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer. Cancer Res 1997;57:5493–7.
9. Carey AH, Waterworth D, Patel K, et al. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene *CYP17*. Hum Mol Genet 1994;3:1873–6.
10. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome *P*450c17alpha gene (*CYP17*) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res 1998;58:585–7.
11. Nativelle-Serpentini C, Lambard S, Séralini GE, Sourdaine P. Aromatase and breast cancer: W39R, an inactive protein. Eur J Endocrinol 2002;146:583–9.
12. Crofts F, Taioli E, Trachman J, et al. Functional significance of different human *CYP1A1* genotypes. Carcinogenesis 1994;15:2961–3.
13. Kisselev P, Schunck WH, Roots I, Schwarz D. Association of CYP1A1 polymorphisms with differential metabolic activation of 17beta-estradiol and estrone. Cancer Res 2005;65:2972–8.
14. Li DN, Seidel A, Pritchard MP, Wolf CR, Friedberg T. Polymorphisms in *P*450 CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. Pharmacogenetics 2000;10:343–53.

15. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 1996;6:243–50.
16. Lee SG, Joo Y, Kim B, et al. Association of Ala72Ser polymorphism with COMT enzyme activity and the risk of schizophrenia in Koreans. Hum Genet 2005;116:319–28.
17. Shen Y, Li DK, Wu J, Zhang Z, Gao E. Joint effects of the CYP1A1 *Msp*I, ERalpha PvuII, and ERalpha XbaI polymorphisms on the risk of breast cancer: results from a population-based case-control study in Shanghai, China. Cancer Epidemiol Biomarkers Prev 2006;15:342–7.
18. Zhang L, Gu L, Qian B, et al. Association of genetic polymorphisms of ER-alpha and the estradiol-synthesizing enzyme genes *CYP17* and *CYP19* with breast cancer risk in Chinese women. Breast Cancer Res Treat. Epub 2008 Jul 16.
19. Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi N, Dahiya R. *CYP1B1* gene polymorphisms have higher risk for endometrial cancer, and positive correlations with estrogen receptor alpha and estrogen receptor beta expressions. Cancer Res 2003;63:3913–8.
20. Garner EI, Stokes EE, Berkowitz RS, Mok SC, Cramer DW. Polymorphisms of the estrogen-metabolizing genes *CYP17* and catechol-*O*-methyltransferase and risk of epithelial ovarian cancer. Cancer Res 2002;62:3058–62.
21. Tanaka Y, Sasaki M, Shiina H, et al. Catechol-*O*-methyltransferase gene polymorphisms in benign prostatic hyperplasia and sporadic prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:238–44.
22. Zhai Y, Qiu W, Dong XJ, et al. Functional polymorphisms in the promoters of MMP-1, MMP-2, MMP-3, MMP-9, MMP-12 and MMP-13 are not associated with hepatocellular carcinoma risk. Gut 2007;56:445–7.
23. Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 2003;19:376–82.
24. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastroenterol Hepatol 1997;12: S294–308.
25. McGlynn KA, London WT. Epidemiology and natural history of hepatocellular carcinoma. Best Pract Res Clin Gastroenterol 2005;19:3–23.
26. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557–76.
27. Huang CS, Chern HD, Chang KJ, Cheng CW, Hsu SM, Shen CY. Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes *CYP17, CYP1A1*, and *COMT*: a multigenic study on cancer susceptibility. Cancer Res 1999;59:4870–5.
28. Yin PH, Lee HC, Chau GY, et al. Polymorphisms of estrogen-metabolizing genes and risk of hepatocellular carcinoma in Taiwan females. Cancer Lett 2004;212:195–201.
29. Yu MW, Chiu YH, Yang SY, et al. Cytochrome *P*450 1A1 genetic polymorphisms and risk of hepatocellular carcinoma among chronic hepatitis B carriers. Br J Cancer 1999;80:598–603.
30. Silvestri L, Sonzogni L, De Silvestri A, et al. CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer. Int J Cancer 2003;104:310–7.
31. Rossi L, Leveri M, Gritti C, et al. Genetic polymorphisms of steroid hormone metabolizing enzymes and risk of liver cancer in hepatitis C–infected patients. J Hepatol 2003;39:564–70.

Lack of Association between the Functional Polymorphisms in the Estrogen-Metabolizing Genes and Risk for Hepatocellular Carcinoma

Xiaoyan Yuan, Gangqiao Zhou, Yun Zhai, et al.

Cancer Epidemiol Biomarkers Prev 2008;17:3621-3627.