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Alcohol, cofactors and the genetics of hepatocellular carcinoma

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Abstract

Heavy alcohol consumption, chronic infection with the hepatitis B virus (HBV) or the hepatitis C virus (HCV), tobacco smoking, and diabetes are risk factors for hepatocellular carcinoma (HCC). In the Los Angeles Non-Asian HCC Study, heavy alcohol intake was shown to exhibit synergistic effects with viral hepatitis (HBV, HCV) and diabetes in the causation of HCC among individuals with joint exposures. Although chronic infection with HBV is recognized as the most important causal factor for HCC in humans, only a minority of HBV carriers eventually develop HCC, suggesting the presence of important cofactors in HBV-related HCC. In the Guangxi/China HCC Study, a 20-fold difference in HCC risk was observed between individuals possessing the least versus the most favorable cytokine genotypes for hepatitis B viral clearance. Experimental studies have indicated an important role for one-carbon metabolism in HCC development. In both the Los Angeles and Guangxi studies, low-activity genotypes (reduced enzymatic activities) of methylenetetrahydrofolate reductase (MTHFR) and high-activity genotypes (enhanced enzymatic activities) of thymidylate synthase (TYMS), both of which discourage the misincorporation of uracil into DNA, were shown to be associated with a reduced risk for HCC.

Keywords

alcohol; cofactor; cytokine; diabetes; genotype; hepatocellular carcinoma; methylation; risk factor; tobacco smoking; viral hepatitis

Introduction

Although rare in North America and Western Europe, primary liver cancer is a common malignancy on a worldwide basis. It is the fifth most common cancer in men and the eighth most common cancer in women, comprising 4% of all newly diagnosed cancers in both sexes combined.¹ The dominant form of primary liver cancer is hepatocellular carcinoma (HCC). Most other primary liver cancers are cholangiocarcinomas, which are histologically and etiologically distinct from HCC. In most regions at high risk for primary liver cancer, including East/South-East Asia and sub-Saharan Africa, over 90% of primary liver cancers are HCC.² Exceptions are areas with high infection rates of liver flukes, which are established causative agents of cholangiocarcinoma. In North-East Thailand where such infections are endemic, 90% of primary liver cancers are cholangiocarcinomas.³ Relative to regions with high HCC incidence, a lower proportion of HCC is seen among cases of primary liver cancer in regions

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The present paper summarizes the findings of three recent epidemiological studies: Yuan *et al.* (2004), Nieters *et al.* (2005), and Yuan *et al.* (2007).

with low HCC incidence. For example, in the USA, 70–75% of primary liver cancer cases are HCC.²

Established or probable risk factors for HCC include chronic infections with the hepatitis B virus (HBV) and/or hepatitis C virus (HCV), intake of aflatoxin-contaminated foods, heavy alcohol consumption, tobacco smoking, history of diabetes, and sustained use of oral contraceptives in low-risk women in the West. There is strong laboratory and epidemiological evidence linking dietary anti-oxidants, specifically retinoids, carotenoids and selenium, to protection against HCC development. A review can be found in Yu and Yuan.⁴

The present article focuses on three recent epidemiological studies reporting on: (i) factors that interact synergistically with heavy alcohol intake in the causation of HCC among individuals with joint exposures; (ii) the effect of cytokine genotypes on HCC risk in a population with virtually all cases of HCC being hepatitis B carriers; and (iii) the effect of methylation genotypes on HCC risk in two populations at polar ends of the HCC risk spectrum.

Alcohol, cofactors and HCC risk

The Los Angeles Non-Asian HCC Study is a population-based, case–control study of newly diagnosed HCC in black, Hispanic, and non-Hispanic white residents of Los Angeles County, who were aged 18–74 years at diagnosis. Cases were identified through the Los Angeles County Cancer Surveillance Program, a population-based cancer registry that records all incident cancers diagnosed in the county of Los Angeles, California, USA. For each interviewed case, up to two control subjects, matched to the index case by gender, age (within 5 years), and race (Hispanic white, non-Hispanic white, black) were recruited from the neighborhood of residence of the index case at the time of cancer diagnosis. All study subjects were interviewed in-person by a trained interviewer using a structured questionnaire that solicited information on lifetime use of tobacco and alcohol, medical history, and other lifestyle-related exposures. Peripheral blood samples were collected from study subjects beginning in January 1992.⁵

Chronic infections with HBV and/or HCV, heavy alcohol drinking, tobacco smoking, and diabetes were statistically significant, independent risk factors for HCC in this relatively low-risk population.⁵ One hundred and thirty-six (55.5%) out of 245 cases were positive for HBV and/or HCV serological markers indicative of either a history of past primary infection or the chronic carrier state. Table 1 shows the pair-wise interactions of diabetes, heavy alcohol drinking, and tobacco smoking on risk of HCC, while Table 2 presents the interactive effects of viral hepatitis with each of the three non-viral risk factors. Both viral hepatitis and diabetes interacted synergistically (i.e. the combined effect greater than the sum of individual effects) with heavy alcohol intake. In fact, except for tobacco smoking, all other independent risk factors interacted in a synergistic fashion in their combined effects on HCC risk. Given the positive correlations of these lifestyle-related factors among study subjects, the present study's findings have both clinical and public health implications for HCC prevention in the USA.

Effect of cytokine genotypes on risk of HBV-related HCC

Although chronic infection with HBV is recognized as the most important causal factor for HCC in humans, only a minority of HBV carriers eventually develop HCC, suggesting the presence of important cofactors in HBV-related HCC. One major goal of the Guangxi/China HCC Study is the elucidation of the role of cytokine genotypes in HCC development in this hyper-endemic region where virtually all cases of HCC arise from chronic HBV carriers.⁶ We postulated that T-helper 1 (Th1) cytokines, specifically γ -interferon (IFN- γ), interleukin-12 (IL-12) and IL-18, which promote hepatitis B viral clearance, are associated with HCC risk reduction. Th2 cytokines, including IL-4 and IL-10, however, were postulated to be associated with HCC risk enhancement, as they suppress Th1 production.

The Guangxi/China HCC Study is a hospital-based, case-control study of newly diagnosed HCC from four major hospitals in the city of Nanning in southern Guangxi, China. For each enrolled HCC case, we identified a consenting control patient among all patients who were admitted to the same hospital within 1 month of the index case's hospital admission, who was without a history of cancer or liver cirrhosis and satisfying the following matching criteria: age (within 3 years), gender, ethnicity (Han, Zhuang, Yao, other), and district/township of residence. All subjects were interviewed in-person by a trained interviewer using a structured questionnaire that solicited information on lifetime use of tobacco and alcohol, and other lifestyle-related exposures. Blood specimens were collected from all subjects. A total of 250 cases and 250 controls were recruited to the study.⁷

Table 3 presents the distributions of Th1 and Th2 genes among study cases and controls. Relative to the putative high-activity genotypes, each individual low-activity genotype of Th1 genes (*IFN-γ*, *IL-12*, *IL-18*) was associated with a statistically non-significant (40–60%) increase in the risk of HCC. More importantly, there was a statistically significant increase in the risk of HCC with increasing number of low-activity genotypes across these three Th1 genes. Similarly, as predicted, individual putative low-activity genotypes of *IL-4* and *IL-10* were associated with reduced risks of HCC. When the numbers of low-activity genotypes were summed across the two Th2 genes, there was a statistically significant decrease in the risk of HCC with increasing number of low-activity Th2 genotypes.

Table 4 shows the combined effect of Th1 and Th2 genotypes on the risk of HCC. Individuals with the maximum number (i.e. three) of low-activity Th1 genes and the minimum number (i.e. zero) of low-activity Th2 genes exhibited an HCC risk level that was 20-fold higher than those with zero low-activity Th1 genes and at least one low-activity Th2 gene.

To our knowledge, this is the first study examining the role of genetic polymorphisms of major Th1 and Th2 cytokine genes in the development of HCC. Our results suggest a major role for genetically regulated immune response to the hepatitis B virus infection in determining an infected individual's level of HCC risk. These findings have major clinical implications, given the severe side-effects of many current antiviral therapies.

Methylation genotypes and HCC risk

Experimental studies have indicated an important role for one-carbon metabolism in HCC development. A schematic representation of one-carbon metabolism is shown in Fig. 1. Rodents with diets deficient in methyl groups (folate, methionine and choline) develop HCC along with increased level of uracil in DNA and accumulated DNA strand breaks in the *p53* gene of hepatocytes.^{8–12} Thymidylate is a rate-limiting nucleotide required for DNA synthesis and repair, and a sufficient pool of thymidylate is essential in minimizing the misincorporation of uracil into DNA, chromosomal breakage and fragile site induction.^{13,14} Thymidylate synthase (TYMS) catalyzes the synthesis of thymidylate, or deoxythymidine monophosphate (dTMP), from deoxyuridine monophosphate (dUMP) with 5,10-methylenetetrahydrofolate as the methyl donor (Fig. 1). Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate. Thus, a lower MTHFR activity would result in a larger pool of 5,10-methylenetetrahydrofolate for TYMS, resulting in increased thymidylate for optimal DNA synthesis and repair. We postulated that reduced MTHFR activity and enhanced TYMS activity may be associated with HCC risk reduction. We tested this hypothesis using both the Los Angeles Non-Asian HCC Study and the Guangxi/China HCC Study databases.¹⁵

Table 5 shows the individual as well as the combined effects of MTHFR and TYMS genotypes on risk of HCC, separately for the Los Angeles and Guangxi studies and for the two databases

combined. As predicted, individual low- versus high-activity MTHFR genotypes and individual high- versus low-activity TYMS genotypes were associated with a 30–50% reduction in the risk of HCC. More importantly, when HCC risk was examined according to the sum of low-activity MTHFR alleles and high-activity TYMS alleles (range, 0–4), a statistically significant, inverse association was observed. Individuals possessing the most favorable genotypes experienced half the level of HCC risk relative to those with the least favorable genotypes (Table 5).

It is interesting to note that it is biologically plausible to link the low-activity genotype of MTHFR to an increased risk of HCC, on the basis that reduced MTHFR activity can reduce the *S*-adenosylmethionine (SAME) pool, especially in the presence of deficient folate, and that chronic hepatic SAME deficiency promotes HCC in animals.¹⁶ In contrast, as we mentioned earlier, reduced MTHFR also promotes DNA synthesis and repair. Although the levels of plasma folate among residents of Guangxi, China are unknown, population-based data on other rural residents of southern China indicate comparable values to those of US populations.¹⁷ Therefore, our study suggests that at least in the presence of relative folate abundance, the DNA synthesis/repair pathway plays a more important role than the methylation pathway in defining the overall effect of MTHFR genotype on HCC risk in humans. However, the role of MTHFR and TYMS polymorphisms on HCC risk remains to be clarified under the setting of relative folate/other methyl group deficiency.

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Biography

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Figure 1.

Schematic representation of one-carbon metabolism. BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; DHF, dihydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; Hcy, homocysteine; MAT, methionine adenosyltransferase; Met, methionine; MS, methionine synthase; MT, methyl transferase; MTA, methylthioadenosine; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; S-AdoMet, S-adenosylmethionine; THF, tetrahydrofolate; TYMS, thymidylate synthase. (Reprinted with permission from Yuan *et al.*¹⁵)

Table 1
Pairwise interactions of diabetes, alcohol drinking, and cigarette smoking on risk of HCC, The Los Angeles Non-Asian HCC Study

Factor 1	Factor 2	Cases	Controls	OR (95% CI) [†]	Interaction effect	
					Additive S (95% CI) [‡]	Multiplicative OR (95% CI) [‡]
Diabetes	Alcohol drinking					
	≤ 4 drinks per day	157	352	1.0		
	> 4 drinks per day	76	45	3.4 (2.2–5.4)		
	Yes	43	36	2.5 (1.5–4.0)		
Diabetes	Alcohol drinking					
	≤ 4 drinks per day	19	2	17.3 (3.9–77.6)		
	> 4 drinks per day				4.2 (2.6–5.8)	2.0 (0.4–10.1)
	Yes					
Diabetes	Cigarette smoking [§]					
	Non-/long-term ex-smokers	127	272	1.0		
	Current-/recent ex-smokers	106	125	1.5 (1.0–2.2)		
	Yes	33	28	2.5 (1.4–4.4)		
Diabetes	Cigarette smoking [§]					
	Non-/long-term ex-smokers	29	10	4.9 (2.2–10.9)		
	Current-/recent ex-smokers				2.0 (0.9–3.1)	1.3 (0.5–3.5)
	Yes					
Diabetes	Alcohol drinking					
	≤ 4 drinks per day	125	276	1.0		
	> 4 drinks per day	35	24	3.3 (1.8–6.1)		
	Yes	75	112	1.5 (1.0–2.2)		
Diabetes	Alcohol drinking					
	> 4 drinks per day	60	23	5.9 (3.3–10.4)	1.7 (0.9–2.6)	1.2 (0.5–2.6)

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[†] Adjusted for age, sex, race, level of education, and all other factors listed in the table.

[‡] Synergy index (S) = (OR₁₁ - 1)/(OR₀₁ + OR₁₀ - 2), where OR₁₁ = odds ratio of the joint effect of the two risk factors; OR₀₁ and OR₁₀ = odds ratio of each risk factor in the absence of the other.

[§] Long-term ex-smokers were those who quit smoking ≥ 10 years ago, and recent ex-smokers were those who quit smoking less than < 10 years ago.

CI, confidence interval; HCC, hepatocellular carcinoma; OR, odds ratio.

Interactions of HBV/HCV infections with diabetes, alcohol drinking, and cigarette smoking on risk of HCC, The Los Angeles Non-Asian HCC Study

Table 2

Factor 1	Factor 2	Cases	Controls	OR (95% CI) [†]	Interaction effect	
					Additive S ₁ (95% CI) [‡]	Multiplicative OR (95% CI) [‡]
HBV/HCV markers	Diabetes					
	Negative	83	179	1.0		
	Yes	26	16	3.2 (1.5–6.7)		
HBV/HCV markers	Alcohol drinking					
	Negative	116	24	8.6 (4.9–14.9)		
	Yes	20	1	47.8 (6.0–377.5)	4.8 (2.7–6.9)	1.8 (0.2–16.3)
HBV/HCV markers	Alcohol drinking					
	≤ 4 drinks per day	80	173	1.0		
	> 4 drinks per day	29	22	2.6 (1.3–5.1)		
HBV/HCV markers	Alcohol drinking					
	Negative	85	23	8.1 (4.6–14.4)		
	Yes	51	2	48.3 (11.0–212.1)	5.5 (3.9–7.0)	2.3 (0.5–12.1)
HBV/HCV markers	Cigarette smoking					
	Negative	62	139	1.0		
	Current-/recent ex-smokers [§]	47	56	1.7 (1.0–3.0)		
HBV/HCV markers	Cigarette smoking					
	Negative	68	17	9.1 (4.7–17.6)		
	Current-/recent ex-smokers	68	8	15.0 (6.4–34.9)	1.6 (0.6–2.6)	1.0 (0.3–2.9)

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[†] Adjusted for age, sex, race, level of education, and all other factors listed in the table.

[‡] Synergy index (S) = (OR₁₁ - 1)/(OR₀₁ + OR₁₀ - 2), where OR₁₁ = odds ratio of the joint effect of the two risk factors; OR₀₁ and OR₁₀ = odds ratio of each risk factor in the absence of the other.

[§] Positive for any one of the three serological markers of HBV and HCV infections: HBsAg, anti-HBc, and anti-HCV.

[¶] Long-term ex-smokers were those who quit smoking 10 or more years ago, and recent ex-smokers were those who quit smoking less than 10 years ago.

anti-HBc, antibody to hepatitis B core antigen; anti-HCV, antibody to hepatitis C virus; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OR, odds ratio.

Table 3

Th1 and Th2 genotypes in relation to HCC risk, The Guangxi/China HCC Study

Genotypes	Cases	Controls	OR (95% CI) [†]	Adjusted OR (95% CI) [‡]
Th1 genes				
<i>IFN-γ</i>				
TT	155	164	1.00	1.00
AT/AA [§]	94	86	1.15 (0.80–1.66)	1.63 (0.82–3.22)
<i>IL-12</i>				
AA	56	72	1.00	1.00
AC/CC [§]	193	178	1.46 (0.93–2.27)	1.37 (0.68–2.75)
<i>IL-18</i>				
GG	172	192	1.00	1.00
GC/CC [§]	77	58	1.48 (0.99–2.20)	1.60 (0.77–3.35)
Total low-activity Th1 genotypes [¶]				
0	27	36	1.00	1.00
1	104	119	1.17 (0.67–2.04)	1.81 (0.69–4.73)
2	94	82	1.58 (0.86–2.91)	1.86 (0.66–5.21)
3	24	13	2.54 (1.08–5.99)	8.94 (1.59–50.23)
<i>P</i> for trend			0.01	0.04
Th2 genes				
<i>IL-4</i>				
CT/TT	243	238	1.00	1.00
CC [§]	6	12	0.46 (0.16–1.31)	0.05 (0.01–0.40)
<i>IL-10</i>				
AA and TT	130	115	1.00	1.00
GG/GA and/or CC/CT [§]	119	135	0.77 (0.54–1.10)	0.56 (0.30–1.05)
Total low-activity Th2 genotypes ^{††}				
0	127	111	1.00	1.00
1	119	131	0.78 (0.54–1.11)	0.48 (0.25–0.92)
2	3	8	0.31 (0.08–1.21)	0.10 (0.01–1.40)
<i>P</i> for trend			0.07	0.01

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CI, confidence interval; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; OR, odds ratio; Th, T helper cell.

Table 4

The combined effect of Th1 and Th2 genotypes on HCC risk, The Guangxi/HCC Study

Total low-activity Th1 genotypes [†]	Total low-activity Th2 genotypes [†]			
	1-2		0	
	Ca/Co [‡]	OR (95% CI) [§]	Ca/Co [‡]	OR (95% CI) [§]
0	16/21	1.00	11/15	2.85 (0.45–18.08)
1	55/58	1.75 (0.48–6.46)	49/61	2.44 (0.75–7.98)
2	41/53	1.16 (0.30–4.49)	53/29	5.13 (1.21–21.81)
3	10/7	7.55 (0.79–72.53)	14/6	19.97 (1.70–234.97)

Reprinted with permission from Nieters *et al.*⁷[†] Low-activity Th1 genotypes were summed across *IFN γ* , *IL12* and *IL18* genotypes; Low-activity Th 2 genotypes were summed across *IL4* and *IL10* genotypes.[‡] Number of cases/number of controls.[§] Adjusted for HBsAg seropositivity and number of alcoholic drinks per day; matching factors were age, sex, and ethnicity.

Table 5

Individual and combined effects of *MTHFR* and *TYMS* genotypes in relation to HCC risk, The Los Angeles Non-Asian HCC Study and The Guangxi/China HCC Study

Genotype	Los Angeles, California		Guangxi, China		Total	
	Ca/Co [†]	OR (95% CI) [‡]	Ca/Co [†]	OR (95% CI) [‡]	Ca/Co [†]	OR (95% CI) [‡]
<i>MTHFR</i> 677						
CC	53/80	1.00	159/156	1.00	212/236	1.00
CT	51/99	0.85 (0.47–1.55)	71/74	0.94 (0.53–1.65)	122/173	0.91 (0.61–1.35)
TT (low-activity)	14/30	0.72 (0.29–1.78)	17/18	0.60 (0.22–1.62)	31/48	0.73 (0.38–1.43)
2-sided <i>P</i> for trend	0.44		0.39		0.36	
<i>MTHFR</i> 1298						
AA	65/104	1.00	136/136	1.00	201/240	1.00
AC	44/85	1.09 (0.60–1.99)	101/91	0.91 (0.54–1.55)	145/176	1.00 (0.68–1.47)
CC (low-activity)	9/20	0.54 (0.19–1.55)	10/21	0.60 (0.20–1.84)	19/41	0.50 (0.23–1.05)
2-sided <i>P</i> for trend	0.50		0.43		0.22	
Sum of <i>MTHFR</i> low-activity alleles						
0	23/26	1.00	77/65	1.00	100/91	1.00
1	49/82	1.07 (0.47–2.46)	114/123	0.82 (0.46–1.48)	163/205	0.87 (0.55–1.37)
2	46/101	0.64 (0.28–1.49)	56/60	0.61 (0.30–1.23)	102/161	0.62 (0.37–1.03)
2-sided <i>P</i> for trend	0.17		0.17		0.06	
<i>TYMS</i> 3'UTR1494						
+6/+6	60/85	1.00	16/14	1.00	76/99	1.00
+6/-6	44/95	0.50 (0.27–0.92)	99/93	0.68 (0.24–1.90)	143/188	0.51 (0.30–0.85)
-6/-6 (putative high-activity)	14/29	0.46 (0.17–1.22)	132/141	0.74 (0.27–2.04)	146/170	0.53 (0.30–0.95)
2-sided <i>P</i> for trend	0.03		0.87		0.07	
Sum of low-activity <i>MTHFR</i> and high-activity <i>TYMS</i> alleles						
0-1	49/55	1.00	42/41	1.00	91/96	1.00
2	41/79	0.44 (0.22–0.86)	96/76	1.02 (0.49–2.12)	137/155	0.68 (0.42–1.10)
3	22/60	0.31 (0.14–0.68)	80/94	0.74 (0.35–1.54)	102/154	0.47 (0.28–0.78)
4	6/15	0.38 (0.10–1.46)	29/37	0.62 (0.25–1.55)	35/52	0.46 (0.23–0.93)
2-sided <i>P</i> for trend	0.005		0.18		0.003	

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[†]No. cases/no. controls.

[‡]Adjusted for age, sex, race/ethnicity, study location (for total subjects only), level of education, number of cigarettes smoked per day, number of alcoholic drinks per day, and hepatitis B/C serology.

CI, confidence interval; *MTHFR*, methylenetetrahydrofolate reductase; OR, odds ratio; *TYMS*, thymidylate synthase.