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Genetic polymorphisms in the cytokine genes and risk of hepatocellular carcinoma in low-risk non-Asians of USA

Simona Ognjanovic[1,2,*], Jian-Min Yuan[2], Ann K.Chaptman[1], Yunhua Fan[2] and Mimi C.Yu[2]

[1]Department of Pediatrics, Division of Pediatric Epidemiology and Clinical Research, University of Minnesota, MMC 715, 420 Delaware Street S.E., Minneapolis, MN 55455, USA and [2]Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, USA

[*]To whom correspondence should be addressed. Tel: +1 612 626 7625; Fax: +1 612 624 7147;
Email: ognja001@umn.edu

Polymorphisms in cytokine genes responsible for inflammatory and immune responses are associated with risk of hepatocellular carcinoma (HCC) in high-risk Chinese population. Similar data in low-risk populations are lacking. A population-based case–control study of HCC was conducted including 120 HCC patients and 230 matched control subjects of non-Asian residents in Los Angeles County, California. Genetic variants in the interferon γ (*IFNγ*), tumor necrosis factor-α (*TNFα*), interleukin-2 (*IL-2*), *IL-4*, *IL-6*, *IL-10*, *IL-12* and *IL-18* genes were determined by Taqman assays. The logistic regression method was used to analyze the data. For T helper (Th) 1 genes (*IFNγ*, *IL-6* and *IL-12*), relative to the putative high-activity genotypes, individual low-activity genotypes were associated with statistically non-significant increases in HCC risk. The odds ratio (OR) was 1.53 [95% confidence interval (CI) = 0.53–4.39] for three versus zero low-activity genotypes. For Th2 cytokines (*IL-4* and *IL-10*), low- versus high-activity genotypes were associated with statistically non-significant decreases in HCC risk. The OR was 0.64 (95% CI = 0.27–1.55) for two versus zero low-activity genotypes. When the Th1 and Th2 genotypes were examined simultaneously, the highest level of risk was observed in individuals jointly possessing the highest number of low-activity Th1 genotypes and the lowest number of low-activity Th2 genotypes. There was a roughly doubling of risk between these two extreme genetic profiles, which did not reach statistical significance (OR = 1.98, 95% CI = 0.50–7.84, *P* = 0.08). In contrast to high-risk Chinese, Th1 and Th2 genotypes did not impact in a major way on risk of HCC in USA non-Asians.

Introduction

Primary liver cancer is the fifth and the eighth most frequent cancer worldwide in men and women, respectively, accounting for 4% of all newly diagnosed cancers in both sexes (1). The dominant form of primary liver cancer is hepatocellular carcinoma (HCC), which is the third most common cause of cancer-related death in the world (1,2). HCC is one of the few types of cancer increasing in frequency and mortality in the USA (3).

While most HCC in high-risk areas can be attributed to hepatitis B virus (HBV) infections and aflatoxin exposure (4–6), only half of HCC cases in the western world can be attributed to the hepatitis viruses (7). In addition, HBV and hepatitis C virus (HCV) infections are equally present among the chronic hepatitis patients in the USA (8). Alcohol and tobacco have been implicated as important causal factors of HCC in western populations. Likewise, there is emerging evidence for obesity and diabetes, two closely related conditions that are on the rise in the USA, as risk factors for HCC and important contributors to the rise in HCC incidence in the USA (9–11). Diabetes- and obesity-related hepatic inflammation, leading to hepatic injury through a series of oxidative stress/lipid peroxidation mediators, has been postulated as the underlying mechanism between diabetes/obesity and hepatocarcinogenesis (12).

Abbreviations: CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFNγ, interferon γ; IL, interleukin; OR, odds ratio; Th, T helper.

It is believed that immune system-mediated chronic inflammation of the liver can lead to HCC development because the former induces continuous cell death, resulting in cell proliferation and increased frequency of genetic alterations (13–15). Thus, ineffective immune response can be a principal oncogenic factor in a chronic HBV- or HCV-infected individual (16). If the T-cell response in an infected individual is strong enough, HBV or HCV can be eliminated from the liver; if not, a procarcinogenic effect can be induced by permanently triggering necroinflammatory disease without resulting in final eradication of HBV or HCV from the liver. The inflammatory response is mediated by cytokines.

Studies of cytokines and their role in cancer development and progression are complicated by pleiotropy and apparent redundancy of cytokine action. Two distinct T helper (Th) cytokine cell subsets, Th1 and Th2, are characterized by distinct and mutually exclusive patterns of cytokine production and different functions (17). Th1 produces *IFNγ*, *IL-2*, *IL-12* and *IL-18* and promote cellular immune response, whereas Th2 produces *IL-4* and *IL-10* and promote humoral response. Th1–Th2 imbalances play an important role in establishment of chronic viral infections in humans (18). A reductionist model proposes that excess Th1 cytokine production leads to a net antiviral state, whereas excess Th2 production tends to counteract the Th1 effect toward a less antiviral state (19). Earlier, we [Nieters *et al.* (20)] proposed that functional variations in cytokine genes associated with viral clearance may play a role in HBV-associated HCC in high-risk Chinese. We showed that individuals with collectively the lowest Th1 and highest Th2 responses experienced a 20-fold increased risk for HCC compared with those with the most favorable genotype combination (high Th1 and low Th2 responses). These results, if confirmed, may open up a potentially new area of risk stratification for HCC (19). The present report describes a comparable study we recently completed among low-risk non-Asians of Los Angeles County, California.

Materials and methods

Participants

The design of the Los Angeles HCC Study has been described previously (12,21). Briefly, cases were newly diagnosed HCC patients of black, Hispanic or non-Hispanic white Americans residing in Los Angeles County who were between 18 and 74 years of age at diagnosis from January 1984 through December 2001. Cases were identified through the Los Angeles County Cancer Surveillance Program, a population-based cancer registry. Due to the rapidly fatal nature of HCC (the median time interval between diagnosis and death is ∼3 months), 84% of eligible patients died prior to our attempted contact. Among the 478 patients we contacted, 34 (7%) were too ill to be interviewed and 325 (73%) of the remaining 444 were interviewed. An experienced hepatopathologist reviewed the histology slides of all interviewed HCC patients; 25 cases judged to be non-HCC were excluded.

For each case, we sought to recruit up to two control subjects from the neighborhoods, where HCC patients resided at the time of cancer diagnosis, who were matched to the index case by sex, age (within 5 years) and race (Hispanic white, non-Hispanic white and black). A total of 474 neighborhood control subjects were recruited into the study, most were the first (74%) or second (12%) eligible neighbors.

All consenting cases and control subjects were interviewed in person by trained interviewers using structured questionnaires, which solicited demographic information, lifetime use of tobacco and alcohol, medical history and other lifestyle factors. We collected from study subjects serum samples beginning in January 1992 and buffy coat samples beginning in October 1995. The buffy coat samples were available on 120 (73%) of 164 eligible HCC cases (i.e. those interviewed after October 1995). For the 277 control subjects from

whom DNA donation was sought, 230 (83%) consented and donated blood samples. We examined and found no differences in the distributions by age, gender, level of education, cigarette smoking, alcohol consumption, history of diabetes and serologic markers for HBV and HCV infections between subjects with DNA (i.e. those included in the present study) and those without DNA, both for the HCC case and control subjects groups.

Laboratory tests

Blood samples from cases and controls were processed and stored (−20°C) in an identical manner. The assays used for testing serologic markers of HBV and HCV infections have been described previously (12,20). Briefly, we tested all study samples for the presence of hepatitis B surface antigen in serum using commercialized kits (AUSRIA, Abbott Laboratories, North Chicago, IL), and negative samples were further tested for the presence of antibodies to the hepatitis B core antigen using standard testing kits (Corab, Abbott Laboratories). All samples were tested for the presence of antibodies to the HCV (anti-HCV) in serum using the ELISA version 2.0 kit manufactured by Ortho Diagnostic Systems (Raritan, NJ), with confirmation of positive samples using RIBA version 2.0 (Chiron, Emeryville, CA). Serum samples were tested blindly, identified only by codes without regard to case–control status.

Genomic DNA was purified from buffy coats using PureGene Blood Kit (Gentra Systems, Minneapolis, MN) or QIAamp96 DNA Blood Kit (Qiagen, Valencia, CA) and utilizing a 96-well robotic platform with silica membrane chemistry (BioRobot 3000; Qiagen). DNA was aliquoted in 96-well plates using robotic equipment to minimize human errors. Genotyping was carried out by the 5′ nuclease Taqman allelic discrimination assay including the Taqman Core Reagent Kit (Applied Biosystems, ABI, Foster City, CA) and specific primers and probes (ABI) according to the manufacturer's instructions. The oligonucleotide primers and probes to detect each of the alleles listed in Table I were either designed by using Primer Express Software (ABI) or commercially available (ABI). Each unique Taqman probe specific for a particular allele was covalently linked to either a 5′ FAM (carboxyfluorescein) or a 5′ VIC (a proprietary die from ABI) reporter dye, whereas 3′ end of each probe was linked to a fluorescent TAMRAquencher dye or a non-fluorescent minor grove binder. Polymerase chain reaction amplifications were performed in a Perkin Elmer GeneAmp PCR System 9600. Each reaction contained template DNA and a 1× Taqman PCR Master Mix [200 μM of each dinucleotide triphosphate, 1× KTE buffer (50 mM KCl, 10 mM Tris–HCl and 0.01 mM ethylenediaminetetraacetic acid), 60 nM passive reference, 5 mM $MgCl_2$, 0.01U/μl AmpErase UNG and 0.025 U/μl AmpliTaq Gold], 300 nM of each Taqman primer, 60 nM wild-type probe and 60 nM variant probe. For each assay, controls included four no template controls, four controls containing the specific target oligonucleotide sequence for one allele and four controls containing the specific oligonucleotide sequence for the second allele. Thermocycling was performed with an initial 50°C incubation for 2 min followed by a 10 min incubation at 95°C. A two-step cycling reaction was performed for 40 cycles, with denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min.

Analysis of the amplification reaction was performed using an ABI Prism 7900 Sequence Detection System (ABI) and Sequence Detection System Software, version 2.0 (ABI). Laboratory personnel was blinded to case–control status. Experimental samples were compared with 12 standard controls identifying genotypes at each locus. Any samples that were outside the parameters were considered non-informative and were retested.

Statistical analysis

The differences between cases and controls in demographic characteristics and in genotype frequencies were analyzed using chi-square test. The unconditional logistic regression models were used to test the association between cytokine gene polymorphisms and HCC risk. The strength of the association was measured by odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) and two-sided *P*-values. The matching factors, age, sex and race (black, Hispanic white and non-Hispanic white), were included in all the logistic regression models. In addition, covariates for known risk factors for HCC were included in the regression models examining the main effects of cytokine genotypes on HCC risk. These covariates included level of education (high school or below, some college, college graduate or above), cigarette smoking (non-/long-term ex-smokers, <20 or 20+ cigarettes/day of current/recent ex-smokers), alcohol drinking (non-drinkers, <3 or 3+ drinks/day), history of diabetes (yes versus no) and hepatitis B/C serology (positive for hepatitis B surface antigen, antibodies to the hepatitis B core antigen or anti-HCV versus negative for all three markers) (12).

Cytokine polymorphisms were chosen based on their influence on the protein expression levels and classified into putatively high or low activity based on their effect on viral clearance. The low activity of Th1 and high activity of Th2 genotypes were hypothesized to increase the risk of HCC development.

Statistical analyses were conducted using SAS software version 9.1 (SAS Institute, Cary, NC). Two-sided *P*-values < 0.05 were considered statistically significant.

Results

Population characteristics are shown in Table II. The mean age at the time of cancer diagnosis was comparable between cases and controls. Compared with controls, cases attained lower level of education, consumed more cigarettes and alcohol, had higher prevalence of hepatitis B and/or C and reported more frequently a history of diabetes.

Genotypic distributions of interferon-γ (*IFNγ*) [both single nucleotide polymorphisms (SNPs)], tumor necrosis factor-α (*TNFα*), interleukin-2 (*IL-2*), *IL-4*, *IL-6*, *IL-10*, *IL-12* and *IL-18* for the case group and control group separately were all in Hardy–Weinberg equilibrium ($P > 0.0028$, a critical *P*-value calculator after Bonferroni adjustments for multiple comparisons).

Table III shows the association between cytokine genotypes and risk of HCC. Compared with the respective high-activity genotypes, low-activity genotypes of individual Th1 genes were associated with modest, statistically non-significant increases in HCC risk. When the total number of putative low-activity Th1 genotypes was examined in relation to risk in an individual, a moderate, gene–dose-dependent positive association was observed, which did not achieve statistical significance. Individuals carrying three versus zero low-activity genotypes had an ∼50% higher risk of HCC (OR = 1.53, 95% CI = 0.53–4.39).

Table III also shows the associations between the Th2 cytokine genotypes and HCC risk. Compared with the respective high-activity genotypes, low-activity genotypes of individual Th2 genes were

Table I. Primers and probes used in Taqman assays to determine polymorphic gene frequencies

Custom Taqman SNP genotyping assays			
Gene	Polymorphism	Primer	Probe
IL-6	−174G/C	FP: 5′-GACGACCTAAGCTGCACTTTTC	5′-VICCGGAAGAAAACATTTMGBNFQ
		RP: 3′-GGGCTGATTGGAAACCTTATTAAGATTG	5′-FAMCGGAAGAAAAGATTTMGBNFQ
IL-12	1188A/C	FP: 5′-GGATCACAATGATATCTTTGCTGTATTT	5′-VICCATTTAGCATCTAACTATACMGBNFQ
		RP: 3′-TGAGAGCTGGAAAATCTATACATAAATTAGC	3′-FAMTTTAGCATCGAACTATACMGBNFQ
IL-18	−137G/C	FP: 5′-GGCACAGAGCCCCAACTTT	3′-VICCGGAAGAAAACATTTMGBNFQ
		RP: 3′-GCAGAGGATACGAGTACTTCTTTTAATG	3′-FAMCGGAAGAAAAGATTTMGBNFQ
ABI predesigned SNP genotyping assays			
Gene	Polymorphism	dbSNP ID	ABI assay ID
IL-2	−330T/G	rs2069762	C_15859930_10
IL-4	−589C/T	rs2243250	C_16176216_10
IL-10	−1082G/A	rs1800896	C_1747360_10
TNFα	−308G/A	rs1800629	C_7514879_10
IFNγ1	IVS3 +284G/A	rs1861494	C_2683477_10
IFNγ2	−764C/G	rs2069707	C_15859751_10

FP, forward primer; RP, reverse primer.

Table II. Distribution of demographic characteristics in the cases and controls, Los Angeles HCC Study

	Cases (n = 120)	Controls (n = 230)	Two-sided P
Age (standard deviation)	60.5 (10.3)	59.5 (10.7)	0.41
Sex (%)			
Males	82 (68.3)	139 (60.4)	0.15
Females	38 (31.7)	91 (39.6)	
Race (%)			
Non-Hispanic whites	71 (59.2)	184 (80.0)	0.0001
Hispanic Americans	40 (33.3)	34 (14.8)	
African-Americans	9 (7.5)	12 (5.2)	
Level of education (%)			
Below high school	23 (19.2)	17 (7.4)	0.0005
High school graduates	33 (27.5)	52 (22.6)	
Some college/occupational school	41 (34.2)	78 (33.9)	
College graduates or above	23 (19.2)	83 (36.1)	
Cigarette smoking (%)			
Never or long-term ex-smokers[a]	70 (58.3)	165 (71.7)	0.01
Current or recent ex-smokers[a]	50 (41.7)	65 (28.3)	
<20 cigarettes/day	15 (12.5)	27 (11.8)	
≥20 cigarettes/day	35 (29.2)	38 (16.5)	
No. of alcoholic drinks per day (%)			
Non-drinkers	35 (29.2)	76 (33.0)	<0.0001
<3	30 (25.0)	113 (49.1)	
≥3	55 (45.8)	41 (17.8)	
Hepatitis B serology (%)			
Negative	85 (70.8)	203 (88.3)	<0.0001
HBsAg or anti-HBc positive	35 (29.2)	27 (11.7)	
Hepatitis C serology (%)			
Anti-HCV negative	62 (51.7)	229 (99.6)	<0.0001
Anti-HCV positive	58 (48.3)	1 (0.4)	
Hepatitis B/C serology (%)			
Both negative	55 (45.8)	203 (88.3)	<0.0001
Either positive	65 (54.2)	27 (11.7)	
History of diabetes (%)			
No	94 (78.3)	207 (90.0)	0.003
Yes	26 (21.7)	23 (10.0)	

HbsAG, hepatitis B surface antigen; anti-HBc, antibodies to the hepatitis B core antigen.
[a]Long-term ex-smokers were those who quit smoking ≥10 years age. Recent ex-smokers were those who quit smoking <10 years ago.

Table III. Distribution of cytokine genotypes in the cases and controls, The Los Angeles HCC Study

Cytokine genotype	Number of cases	Number of controls	OR (95% CI)[a]	Adjusted OR[b] (95% CI)
Th1 genes				
TNFα				
GA/AA	28	49	1.00	1.00
GG[c]	90	176	0.77 (0.44–1.35)	0.88 (0.46–1.68)
IL-2				
TT	58	103	1.00	1.00
TG/GG[c]	58	115	0.91 (0.57–1.45)	0.98 (0.57–1.67)
IL-12				
AA	57	128	1.00	1.00
AC/CC[c]	60	95	1.25 (0.78–2.00)	1.24 (0.72–2.12)
IL-18				
GG	61	111	1.00	1.00
GC/CC[c]	56	105	0.85 (0.53–1.36)	0.98 (0.56–1.69)
IL-6				
GC/CC	46	118	1.00	1.00
GG[c]	71	103	1.44 (0.89–2.34)	1.42 (0.81–2.49)
IFNγ (SNP1)				
GG	48	87	1.00	1.00
GA/AA[c]	66	122	1.04 (0.64–1.69)	0.99 (0.57–1.73)
IFNγ (SNP2)				
GG/GC	57	114	1.00	1.00
CC[c]	56	92	1.28 (0.79–2.06)	1.20 (0.70–2.08)
Total low-activity Th1 genotypes[d]				
0	15	37	1.00	1.00
1	36	83	1.01 (0.48–2.09)	1.22 (0.53–2.84)
2	44	68	1.39 (0.67–2.90)	1.59 (0.69–3.66)
3	19	22	1.30 (0.52–3.25)	1.53 (0.53–4.39)
P for trend			0.30	0.27
Th2 genes				
IL-4				
CT/TT	47	77	1.00	1.00
CC[c]	71	147	1.03 (0.63–1.69)	0.91 (0.51–1.61)
IL-10				
GG/GA	79	147	1.00	1.00
AA[c]	39	67	0.89 (0.53–1.48)	0.80 (0.44–1.45)
Total low-activity Th2 genotypes[e]				
0	27	46	1.00	1.00
1	72	127	1.03 (0.57–1.85)	0.89 (0.45–1.76)
2	18	40	0.85 (0.40–1.82)	0.64 (0.27–1.55)
P for trend			0.70	0.34

[a]ORs were calculated using logistic regression models, adjusting for age, gender and ethnicity (non-Hispanic white or Hispanic white/black).
[b]ORs were further adjusted for level of education, cigarette smoking (never-/long-term ex-smokers, <20 or 20+ cigarettes/day of current/recent ex-smokers), number of alcoholic drinks per day (non-drinkers, <3 or ≥3), history of diabetes and hepatitis B/C serology (positive for hepatitis B surface antigen, antibodies to hepatitis B core antigen or antibodies to HCV).
[c]Putative low-activity genotypes.
[d]Summed across *IL-6*, *IL-12* and *IL-18* genotypes.
[e]Summed across *IL-4* and *IL-10* genotypes.

associated with 10–20% lower risk of HCC. When the number of low-activity genotypes in *IL-4* and *IL-10* were summed in a given subject, a moderate, gene–dose-dependent inverse association with HCC risk was observed (OR = 0.64, 95% CI = 0.27–1.55 for two versus none).

The combined effects of Th1 and Th2 genotypes on HCC risk are shown in Table IV. Within each Th2 genotype category, the number of low-activity Th1 genotypes was associated with increased risk of HCC. Conversely, the number of the low-activity Th2 genotypes was associated with reduced risk of HCC within each Th1 genotype category. In combination, individuals with the highest number of putative low-activity Th1 genotypes (two to three) and the minimum number of putative low-activity Th2 genotypes (zero to one) experienced a doubling of HCC risk (OR = 1.98, 95% CI = 0.50–7.84) compared with individuals without any putative low-activity Th1 genes and the highest number (n = 2) of putative low-activity Th2 genotypes.

Discussion

To our knowledge, this is the first study of cytokine polymorphisms and HCC risk in low-risk non-Asians. In contrast to our earlier findings in high-risk Chinese, in which a 20-fold, statistically significant difference in HCC risk was observed between subjects possessing the least versus most favorable profile of cytokine genotypes, a mere 2-fold, statistically non-significant variation in HCC risk was noted between blacks and whites of Los Angeles County at polar ends of their cytokine profiles.

In this non-Asian population of Los Angeles County, infections with HBV and HCV, heavy smoking and alcohol consumption and a history of diabetes are independent risk factors for HCC (8,11). As most of these risk factors induce inflammation of the liver, we hypothesized that the individuals with genotypes for less-effective immune response, such as low Th1 and high Th2 response genotypes, may have an increased risk of HCC development. Although the associations we observed followed the hypothesized direction, they were very modest and did not reach statistical significance due to the relatively small number of HCC cases. Thus, our study could not reach a definitive conclusion that Th1 and Th2 genetic polymorphisms play a significant role in HCC development among non-Asians in the USA.

Table IV. The combined effect of Th1 and Th2 genotypes on the risk of HCC

Total no. of low-activity Th1 genotypes*	Total no. of low-activity Th2 genotypes*			
	2		0–1	
	Cases/ controls	OR[a] (95% CI)	Cases/ controls	OR[a] (95% CI)
0	4/10	1.00	11/24	1.27(0.28–5.89)
1	6/13	1.32 (0.24–7.41)	30/67	1.46 (0.35–6.03)
2–3	8/16	1.16 (0.21–6.45)	54/71	1.98 (0.50–7.84)

[a]ORs were further adjusted for level of education, cigarette smoking (never-/long-term ex-smokers, <20 or 20+ cigarettes/day of current/recent ex-smokers), number of alcoholic drinks per day (non-drinkers, <3 or ≥3), history of diabetes and hepatitis B/C serology (positive for hepatitis B surface antigen, antibodies to hepatitis B core antigen or antibodies to HCV).

IL-6 plays an important role in liver protection in T-cell-mediated liver injury, including hepatitis (22,23). It inhibits apoptosis (24) and promotes liver regeneration and protects against a multitude of liver-damaging influences, including alcohol (23,25). Its production in the liver is increased by heavy smoking (26). Therefore, in addition to its role in hepatitis viral infections, IL-6 may affect the risk of HCC via non-hepatitis pathway. Several studies, all conducted in Asian populations (Chinese, Japanese and Koreans), attempted to examine the effect of *IL-6* genotype on risk of HCC and failed to do so given the gene's monomorphism in Asians (20,27,28). We are the first to be able to assess the association between *IL-6* genetic polymorphism and HCC risk. Previous study found that the C allele of the *IL-6* genotype is associated with increased protein levels (29). Our study demonstrated that among all the cytokine polymorphisms studied, the low-activity *IL-6* genotype showed the strongest effect on HCC risk. IL-6 is a cytokine that does not strictly conform to the Th1–Th2 classification. We have grouped it with Th1 cytokines when we examined the combined effect of multiple genes based on the observed association between the low-activity genotype and increased risk of HCC.

The role of polymorphism in *IFNγ* (+847T/A) in hepatitis B viral clearance has been most frequently studied. We have shown a small positive association between the AA genotype of *IFNγ* and increased risk of HCC (20). As chronic hepatitis in the USA is equally attributed to HBV and HCV, we have studied two recently reported *IFNγ* polymorphisms, IVS3 +284G/A and −764C/G. The latter has been implicated in HCV clearance (30). We have analyzed these two SNPs together as a haplotype and found no association with HCC risk (OR = 0.98, 95% CI = 0.44–2.14).

Among Th2 cytokines, the polymorphisms in *IL-10* have been extensively studied in HCC. The results are conflicting. The −1082G/A polymorphism associated with reduced plasma levels (AA) (31) was most frequently studied. Shin *et al.* (2003) showed no association for this polymorphism, but a haplotype of *IL-10* (32) leading to highest protein expression was associated with chronic HBV progression to HCC. Likewise, no associations were found by two other studies of HCC risk among chronic HBV carriers (27,33). Large ethnic differences in genotypes distributions for this polymorphism were observed; G allele was present in only 4–6% of Chinese populations (20,34) compared with close to half of participants in this study as well as in whites in the Heneghan *et al.* (34) study. Similar to our earlier observation in Guangxi, China, the present study shows a non-significant decrease in HCC risk associated with low-activity genotypes of *IL-10*. On the other hand, the substantial decrease in HCC risk associated with *IL-4* (−589C/T) polymorphism noted in Guangxi, China, was not present in Los Angeles non-Asians.

Several factors are probably to be responsible for the disparate associations between cytokine genotypes and HCC risk in high-risk Chinese versus low-risk USA blacks and whites. Foremost are the differences between the two populations in terms of the types and time/mode of primary infection of the hepatitis viruses. In Guangxi, China, it has been established that HCV plays a negligible role in HCC development (35) and that vertical transmission from carrier mother to child, occurring during or shortly after birth, is the predominant mode of HBV transmission in this hyperendemic region (36). On the other hand, horizontal transmission occurring during adulthood, relating to lifestyle choices, is the principal route of HBV or HCV infection in low-risk USA blacks and whites (36). Thus, in the USA, hepatitis infections occur in adults with a mature immune system, whereas in China, they occur in infants with a developing immune system. In our opinion, the differences in the natural history of hepatitis infections between USA non-Asians and native Chinese are probably to impact on the roles of cytokines in hepatitis-related HCC development.

Diet may be another cause of the varying findings between USA non-Asians and native Chinese. We and others (36) have reported inverse associations between dietary antioxidants, specifically retinoids and selenium, and HCC risk in prospective population-based cohorts. There is laboratory evidence that dietary antioxidants can stimulate immune system toward antiviral activity (37). Population-based surveys have documented higher levels of exposure to selenium and vitamin A in USA versus native Chinese populations (38–40). In addition, dietary aflatoxin is a recognized major contributor of HCC risk in China, while it is tightly regulated by the Food and Drug Administration in this country, and thus considered to be a minor, if at all, player in contributing to the HCC burden in the USA. It is known that aflatoxins exert immunosuppressive effects (41).

There are limitations to our study. The recruitment rate of cases was low due to the high fatality of this cancer; the average survival time after HCC diagnosis is ∼3 months (Surveillance, Epidemiology, and End Results Program). The vast majority (84%) of cases identified from the cancer registry died before our attempted contact. However, we found no evidence that the patients who participated in the study differed from the eligible patients who died before we approached them for participation. Another limitation of the study is the fact that biospecimens were not collected from controls at the inception of the study. However, we found no significant differences in age, gender, cigarette smoking, alcohol drinking and history of diabetes between controls who donated blood versus those who either refused or were not asked during the first 10 years of the study. Finally, the present study is quite modest in sample size. However, the statistical power of this study to detect comparable main effects of total low-activity Th1 and Th2 that noted in our earlier, Guangxi, China study is 100%. So, we can still note, with some degree of certainty, that the cytokine–HCC association previously noted in native Chinese does not apply to USA blacks and whites.

In summary, the present study shows that genetic polymorphisms in Th1 and Th2 cytokine genes are modestly associated with HCC risk. This contrasts with the strong association noted earlier in native southern Chinese. Differences in the mode of transmission of the viral hepatitises and timing of the primary infections between the two populations may explain the two sets of disparate findings. Dietary cofactors that are known to influence the hepatitis–HCC link, including dietary antioxidants and aflatoxins, may also play a role.

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