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## Androgen-Receptor Gene CAG Repeats, Plasma Testosterone Levels, and Risk of Hepatitis B-Related Hepatocellular Carcinoma

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**Background:** Worldwide, hepatocellular carcinoma (HCC) is more prevalent in men than in women, suggesting that sex hormones and/or X-chromosome-linked genes may be involved in hepatocarcinogenesis. We investigated the association of a trinucleotide (CAG) repeat in the androgen receptor (AR) gene (located on the X chromosome) termed “AR-CAG repeats,” levels of plasma testosterone, and the risk of HCC in Taiwanese men. Chronic hepatitis B virus (HBV) infection, which is associated with risk of HCC, is hyperendemic in Taiwan. **Methods:** We compared the number of AR-CAG repeats in 285 HBV carriers with HCC and in 349 HBV carriers without HCC. We also conducted a nested case-control study on participants in a cohort study. Blood was collected prospectively from 110 case patients and 239 control subjects and was used to determine the number of AR-CAG repeats and plasma testosterone level. All statistical tests were two-sided. **Results:** The overall odds ratio (OR) for HCC was 1.72 (95% confidence interval [CI] = 1.03–2.89) for HBV carriers with 20 or fewer AR-CAG repeats compared with those with more than 24 repeats. This association was observed only in patients with late-onset HCC (OR = 2.37; 95% CI = 1.28–4.38). In the nested case-control study, HBV carriers in the highest tertile of testosterone levels had a statistically significantly increased risk of HCC (OR = 2.06; 95% CI = 1.14–3.70) compared with those in the lowest tertile. Elevated testosterone was more strongly associated with early-onset (OR = 4.67; 95% CI = 1.41–15.38) than late-onset disease. HBV carriers with 20 or fewer AR-CAG repeats and

higher testosterone levels had a four-fold increase in HCC risk compared with those with more than 24 repeats and testosterone levels in the lowest tertile. **Conclusions:** Higher levels of androgen signaling, reflected by higher testosterone levels and 20 or fewer AR-CAG repeats, may be associated with an increased risk of HBV-related HCC in men. [J Natl Cancer Inst 2000;92:2023–8]

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Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) has a major role in the development of the disease (1), and HCC is more prevalent in men than in women throughout the world (1). In Taiwan, where chronic HBV infection is hyperendemic, the HCC incidence rate for men is approximately three times that for women, despite similar rates of chronic HBV infection (2). This difference between the sexes may be, at least in part, attributable to differences in exposure to risk factors for HCC, such as alcohol consumption and cigarette smoking (3,4). However, sex hormone and X-chromosome-linked genetic factors may also be important. Abundant data show the association of testosterone levels and hepatocarcinogenesis in animal experiments (5–11). In humans, although numerous case reports have suggested that therapeutic use of androgenic steroids may induce HCC (12,13), data on the role of endogenous male hormones in hepatocarcinogenesis are limited, and an asso-

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ciation between the risk of HCC and pre-diagnostic levels of serum testosterone remains controversial (14,15).

Hormonal signals are transmitted through hormone receptors. The androgen receptor (AR) gene, located on the long arm of the X chromosome, encodes AR protein, which acts as a nuclear transcriptional activator when bound to androgens (16). An association between the number of polymorphic exon 1 CAG repeats in the transactivation domain of the AR gene and a predisposition for cancer has been reported. AR genes with fewer CAG repeats were associated with an increased risk of prostate cancer in men (16–18) but a decreased risk of BRCA1-associated breast cancer in women (19). The number of CAG repeats is reflected in the number of glutamine residues [poly(Q)] in the amino-terminal domain of the AR protein. *In vitro* studies have demonstrated an inverse relationship between AR transactivation activity and the number of CAG repeats (20–22), which is related to the increased ability of longer poly(Q) regions to inhibit the interaction between AR and coactivators (22).

Both HCC and nontumorous liver tissue contain ARs (23). In mice, functional ARs are required for testosterone to promote hepatocarcinogenesis (11). Increased hepatic AR expression was found in female rats during development of chemically induced HCC (24). These findings suggest that the effect of testosterone on the development of human HCC may be AR dependent. Therefore, it is possible that polymorphisms in the number of AR-CAG repeats may have a role in modifying the pathogenesis of HCC.

In this study, we tested this hypothesis on the data from our large cohort study, conducted among men chronically infected with HBV, and also a series of male patients with HBV-related HCC, recruited on a comprehensive basis in Taiwan. We simultaneously evaluated whether there was an association of the risk of HCC, pre-diagnostic plasma levels of testosterone, and the number of CAG repeats in the AR gene.

## SUBJECTS AND METHODS

### The Cohort

The cohort consisted of 4841 male HBV carriers aged 30 years or older who attended the specific clinic for asymptomatic HBV carriers at the Liver Unit of Chang-Gung Memorial Hospital (Taipei, Taiwan) and the Government Employee Central

Clinics (Taipei, Taiwan) for regular health examinations from 1988 through 1992 (3,25). An HBV carrier is defined as an individual who tests positive for the hepatitis B surface antigen (HBsAg). Written informed consent was obtained from all study participants, and the investigation was approved by the research ethics committee at the College of Public Health, National Taiwan University, Taipei, and the appropriate institutional review board. After an initial baseline examination, an in-person interview was conducted with the use of a structured questionnaire to obtain information on demographic characteristics, lifetime habits of alcohol and tobacco use, as well as personal and family histories of major chronic diseases. Blood specimens, including white blood cells, serum, and plasma, were also obtained and frozen at  $-70^{\circ}\text{C}$  until subsequent analysis. Participants were monitored for incident cancer through various channels, including periodic ultrasonography measurement and conventional liver function tests every 6–12 months, a personal telephone interview, abstraction of their medical records, and a data linkage to the national death certification and cancer registry systems. After 11 years of follow-up, approximately 70% of the HBsAg carriers surviving continued to return for follow-up examination. By September 30, 1999, we had confirmed 138 incident cases of HCC and had sufficient DNA samples from 112 of these patients for the AR genetic polymorphism assay. HCC was diagnosed on the basis of histologic findings or an elevated level of serum  $\alpha$ -fetoprotein ( $\geq 400$  ng/mL) combined with at least one positive image from angiography, sonography, and/or computed tomography. On average, 4.3 years elapsed between blood collection and diagnosis of HCC.

Three hundred forty-nine control subjects were selected for the 112 case patients (control subject/case patient ratio = one to seven control subjects per case patient), matched to case patients for date of blood collection (within 3 months) and year of birth (within 5 years, except for one elderly case patient who was matched within 10 years). The control subjects were randomly selected from cohort subjects with available DNA samples who were alive and remained unaffected with HCC throughout the follow-up period.

### Recruitment of Hospital-Based Case Patients

To increase the statistical power for detecting a statistically significant association between the number of AR-CAG repeats and the risk of HCC, we also selected patients with newly diagnosed HCC from male HBV carriers with HCC who participated in our ongoing genetic epidemiology study of HCC. These patients were consecutively recruited from three major hospitals (Chang-Gung Memorial Hospital, National Taiwan University Hospital, and Taipei Veterans General Hospital) in Taipei City, Taiwan. On average, fewer than 5% of the hospital patients meeting the diagnostic criteria who were approached for interview refused to participate. The first 175 male HBV carriers with newly diagnosed HCC who gave written informed consent for collection of blood samples were included in this study. Two case patients were excluded because the polymerase chain reaction failed to amplify their DNA, leaving 173 case patients in our analysis.

Thus, we had a total of 285 case patients with

HCC, 112 from the cohort study and 173 from the hospital-based group.

### Laboratory Analysis

The status of serum HBsAg was determined by a radioimmunoassay (Abbott Laboratories, Chicago, IL). DNA was purified from peripheral white blood cells. The CAG trinucleotide repeat found in exon 1 of the AR gene was amplified, and the number of repeats was determined as described previously (18). Plasma testosterone levels were measured by a competitive immunoassay with the use of direct chemiluminescent technology (Chiron Diagnostics Corp., East Walpole, MA). Because the onset of HCC may affect the plasma level of testosterone, testosterone was not measured in case patients recruited from a hospital after diagnosis of liver cancer. Because of our limited budget, we tested plasma testosterone in all cohort-based case patients who had sufficient frozen plasma samples (110 case patients) but only a random sample of matched control subjects (239 control subjects). These assays were conducted by laboratory personnel blinded to case-control status.

### Statistical Analysis

Since there is no *a priori* point at which cutoffs may be applied to identify allele-specific risk groups, the numbers of AR-CAG repeats were originally categorized as deciles based on the distribution among control subjects. Cut points were chosen after combining categories with similar risks. Unconditional logistic regression was used to compute the odds ratios (ORs) and their 95% confidence intervals (CIs). Because the AR gene is inherited maternally and the case patients and control subjects were not matched on maternal ethnicity, the ORs of HCC associated with various numbers of AR-CAG repeats were adjusted for year of birth (continuous variable) and maternal ethnicity (Fukien Taiwanese, Hakka Taiwanese, and Mainland Chinese). In the case-control study nested within the cohort study, subjects were divided into tertiles of plasma testosterone levels based on the distribution among the control group. Tertiles of testosterone values were chosen as cut points to have a sufficient number of case patients and control subjects in each cell so that both the independent effect of testosterone and its interactive effect with the number of AR-CAG repeats could be determined. Age, alcohol consumption, chronic liver disease, and cigarette smoking are HCC risk factors that might be associated with blood testosterone levels (26,27). Multivariate-adjusted ORs of HCC associated with testosterone levels were thus computed after adjustment for matching variables (e.g., year of birth and the time that blood was drawn) and potential confounders, including alcohol drinking, cigarette smoking, a history of chronic liver disease, and educational level (senior high school and above, junior high school, or primary school and below). For univariate analyses, Mantel's  $\chi^2$  test for a trend was used to assess the dose-response relationship. For multivariate analyses, tests for linear trends were performed in logistic regression by assigning the medians of each category as scores. In addition to total HCC, we conducted analyses stratified by age at diagnosis of HCC. The cut point between early- and late-onset HCC, 50 years of age, was as defined in our previ-

ous study of familial aggregation. In this study, the cumulative HCC risk to first-degree relatives was greater for case patients diagnosed at less than 50 years of age than for those diagnosed at 50 years of age or older (28). The effect modifying the relationship of testosterone and HCC by the AR genotype was examined by adding interaction terms (genotype and testosterone) to a logistic regression model and computing the likelihood ratio statistic. All *P* values were from two-tailed tests.

## RESULTS

The mean age at diagnosis of cancer was  $55.3 \pm 9.3$  years ( $\pm$  standard deviation; range = 36–79 years) for cohort-based case patients and  $53.3 \pm 9.9$  years (range = 36–83 years) for hospital-based case patients (*P* = .091). The cohort-based case patients and hospital-based case patients were similar with respect to the distribution of the number of AR-CAG repeats (*P* = .399) (Table 1). To gain statistical power, we thereafter combined the two groups of case patients in

the analyses performed to assess the independent effect of the AR polymorphism on HCC risk.

The number of CAG repeats ranged from 14 to 31 among case patients (median = 22 repeats) and from 15 to 35 among control subjects (median = 23 repeats). Compared with male HBsAg carriers who had more than 24 CAG repeats, those with 20 repeats or fewer had an overall OR of 1.72 (95% CI = 1.03–2.89; *P* = .040) for HCC after adjustment for year of birth and maternal ethnicity (Table 2). AR genes with more than 24 CAG repeats were found in 40.2% (39 of 97) of case patients diagnosed with HCC before age 50 years but in only 21.8% (41 of 188) of case patients diagnosed at age 50 years or older. The difference in the proportion of subjects with more than 24 CAG repeats between the two groups of patients was statistically significant, even after adjusting for maternal ethnicity (*P*

= .001). Subsequent stratification according to age at diagnosis of cancer showed that AR genes with 20 CAG repeats or fewer were a statistically significant risk factor for late-onset HCC diagnosed at age 50 years or older (multivariate-adjusted OR = 2.37; 95% CI = 1.28–4.38; *P* = .006) but not for early-onset HCC.

Using data from the prospectively collected plasma samples from 110 case patients and 239 control subjects within the cohort study of 4841 male HBsAg carriers, we examined the relation between plasma testosterone levels and HCC risk (Table 3). A strong trend of increasing risk of HCC was observed with increasing levels of plasma testosterone (multivariate-adjusted ORs by tertile = 1.00 [referent]; 1.09 [95% CI = 0.57–2.07], and 2.06 [95% CI = 1.14–3.70]; *P*<sub>for trend</sub> = .009). To investigate further the association between plasma testosterone level

**Table 1.** Distribution of the number of CAG repeats in the androgen receptor (AR) gene in hepatitis B surface antigen (HBsAg)-positive case patients and control subjects\*

	No. of AR-CAG repeats			
	>24	23–24	21–22	≤20
No. (%) of control subjects (n = 349)	111 (31.8)	88 (25.2)	112 (32.1)	38 (10.9)
Cohort-based HCC case patients (n = 112)				
No. (%)	29 (25.9)	26 (23.2)	41 (36.6)	16 (14.3)
OR (95% CI)	1.00†	1.13 (0.62–2.06)	1.40 (0.81–2.41)	1.61 (0.79–3.29)
Hospital-based HCC case patients (n = 173)				
No. (%)	51 (29.5)	44 (25.4)	47 (27.2)	31 (17.9)
OR (95% CI)	1.00†	1.09 (0.67–1.78)	0.91 (0.57–1.47)	1.78 (1.00–3.17)

\*HCC = hepatocellular carcinoma; OR = odds ratio; CI = confidence interval.

†Referent.

**Table 2.** Frequency distribution of the number of CAG repeats in the androgen receptor (AR) gene among early- and late-onset hepatitis B surface antigen (HBsAg)-positive case patients with hepatocellular carcinoma (HCC) compared with HBsAg-positive control subjects\*

Characteristic	No. of AR-CAG repeats				<i>P</i> <sub>for trend</sub> †
	>24	23–24	21–22	≤20	
No. of control subjects	111	88	112	38	
Total No. of case patients	80	70	88	47	
Univariate OR (95% CI)	1.00‡	1.10 (0.72–1.69)	1.09 (0.73–1.63)	1.72 (1.03–2.87)	.097
Adjusted OR§,   (95% CI)	1.00‡	1.11 (0.72–1.70)	1.08 (0.72–1.61)	1.72 (1.03–2.89)	.104
No. of early-onset case patients¶	39	16	29	13	
Univariate OR (95% CI)	1.00‡	0.52 (0.27–0.99)	0.74 (0.43–1.27)	0.97 (0.47–2.02)	.639
Adjusted OR§,   (95% CI)	1.00‡	0.56 (0.27–1.17)	0.86 (0.46–1.63)	1.13 (0.49–2.63)	.978
No. of late-onset case patients	41	54	59	34	
Univariate OR (95% CI)	1.00‡	1.66 (1.01–2.72)	1.43 (0.88–2.30)	2.42 (1.35–4.35)	.011
Adjusted OR§ (95% CI)	1.00‡	1.63 (0.97–2.73)	1.27 (0.77–2.10)	2.37 (1.28–4.38)	.029

\*OR = odds ratio; CI = confidence interval.

†All *P* values are from two-sided tests.

‡Referent.

§Adjusted for year of birth (continuous variable) and maternal ethnicity (Fukien Taiwanese, Hakka Taiwanese, and Mainland Chinese).

||One case patient was excluded from analysis because of missing data on maternal ethnicity.

¶Case patients who were diagnosed at younger than 50 years.

**Table 3.** Risk of hepatocellular carcinoma (HCC) by tertile of baseline plasma testosterone levels among 110 hepatitis B surface antigen (HBsAg)-positive case patients and 239 HBsAg-positive control subjects nested within the Taiwan HCC cohort study\*

	Tertile testosterone, ng/mL			<i>P</i> <sub>for trend</sub> †
	Lowest (0.87–4.73)	Middle (4.74–6.38)	Highest (6.39–13.99)	
All case patients versus all control subjects				
No. of case patients/No. of control subjects	25/79	27/80	58/80	
Univariate OR (95% CI)	1.00‡	1.07 (0.57–2.00)	2.29 (1.31–4.02)	.002
Adjusted OR§,   (95% CI)	1.00‡	1.09 (0.57–2.07)	2.06 (1.14–3.70)	.009
Early-onset case patients¶ versus their matched control subjects				
No. of case patients/No. of control subjects	5/28	6/27	24/30	
Univariate OR (95% CI)	1.00‡	1.24 (0.34–4.56)	4.48 (1.50–13.36)	.002
Adjusted OR§,   (95% CI)	1.00‡	1.83 (0.45–7.35)	4.67 (1.41–15.38)	.007
Late-onset case patients versus their matched control subjects				
No. of case patients/No. of control subjects	20/51	21/53	34/50	
Univariate OR (95% CI)	1.00‡	1.01 (0.49–2.08)	1.73 (0.88–3.41)	.095
Adjusted OR§ (95% CI)	1.00‡	1.02 (0.49–2.15)	1.64 (0.81–3.32)	.132

\*OR = odds ratio; CI = confidence interval.

†All *P* values are from two-sided tests.

‡Referent.

§Adjusted for year of birth (continuous variable), the time of blood draw (continuous variable), alcohol consumption, cigarette smoking, history of chronic liver disease, and educational levels (senior high school and above, junior high school, or primary school and below).

||One case patient was excluded from analysis because of missing data on educational levels, habits of cigarette smoking and alcohol consumption, and history of chronic liver disease.

¶Case patients diagnosed at younger than 50 years.

and HCC risk, we repeated the basic analyses including only those case-control matched sets in which the case patients were diagnosed 4 years or more after the start of follow-up. With the remaining 62 case patients and 140 control subjects, we observed results similar to those from previous analyses with all of the case patients and control subjects (multivariate-adjusted OR = 2.53; 95% CI = 1.13–5.66 for the highest versus the lowest tertile; *P*<sub>for trend</sub> = .016). Elevated

plasma testosterone levels were more strongly associated with early-onset disease than with late-onset disease.

Data in Table 4 show the effect of the combined contributions of the number of AR-CAG repeats and the level of testosterone to the risk of developing HCC. Although we observed no statistically significant interaction between testosterone and AR-CAG repeats (*P* = .24), the association between the level of testosterone and HCC risk appeared stronger for

male HBsAg carriers with 20 CAG repeats or fewer, although not statistically significantly so (*P* = .24 for the interaction). Male HBsAg carriers with 20 CAG repeats or fewer in the highest tertile of testosterone had a multivariate-adjusted OR of 8.32 (95% CI = 0.86–80.81; *P* = .068) compared with those who had a similar number of CAG repeats in the lowest tertile of testosterone. The comparable multivariate-adjusted OR among male HBsAg carriers with more than 24

**Table 4.** Risk of hepatocellular carcinoma (HCC) by the number of CAG repeats in the androgen receptor (AR) gene and tertile of baseline plasma testosterone levels among 110 hepatitis B surface antigen (HBsAg)-positive case patients and 239 HBsAg-positive control subjects nested within the Taiwan HCC cohort study\*

Tertile testosterone	No. of AR-CAG repeats		
	>24	24–21	≤20
Lowest			
No. of case patients/No. of control subjects	8/27	15/41	2/11
Univariate OR (95% CI)	1.00†	1.24 (0.46–3.31)	0.61 (0.11–3.36)
Adjusted OR‡,§ (95% CI)	1.00†	1.36 (0.49–3.81)	0.75 (0.13–4.30)
Middle			
No. of case patients/No. of control subjects	2/19	22/54	3/7
Univariate OR (95% CI)	0.36 (0.07–1.86)	1.38 (0.54–3.49)	1.45 (0.30–6.92)
Adjusted OR‡,§ (95% CI)	0.40 (0.07–2.17)	1.44 (0.54–3.84)	2.12 (0.42–10.63)
Highest			
No. of case patients/No. of control subjects	18/26	29/47	11/7
Univariate OR (95% CI)	2.34 (0.87–6.30)	2.08 (0.83–5.20)	5.30 (1.55–18.20)
Adjusted OR‡,§ (95% CI)	2.70 (0.95–7.69)	1.92 (0.73–5.06)	4.09 (1.10–15.24)

\*OR = odds ratio; CI = confidence interval.

†Referent.

‡Adjusted for year of birth (continuous variable), the time of blood draw (continuous variable), alcohol consumption, cigarette smoking, history of chronic liver disease, educational levels (senior high school and above, junior high school, or primary school and below), and maternal ethnicity (Fukien Taiwanese, Hakka Taiwanese, and Mainland Chinese).

§One case patient was excluded from analysis because of missing data on maternal ethnicity, habits of cigarette smoking and alcohol consumption, history of chronic liver disease, and educational levels.

repeats was 2.70 (95% CI = 0.95–7.69;  $P = .063$ ). Male HBV carriers in the highest testosterone tertile and 20 AR-CAG repeats or fewer had an increased risk of HCC that was approximately fourfold higher than male HBV carriers in the lowest testosterone tertile and more than 24 AR-CAG repeats.

## DISCUSSION

Most HBV carriers in Taiwan are infected by the virus during their early childhood through vertical transmission but are usually not affected with HCC until several decades after infection (2). Although there has been a great deal of progress in elucidating the risk factors associated with the development of HCC (1,3,4,25,28,29), our understanding of the molecular mechanisms of HCC remains rudimentary. The predominance of males with HCC has long been observed in humans and various animal models, including HBV-transgenic mice (1,2,5–8,10,11,30,31). Castration of male mice decreased the incidence of chemically induced HCC compared with that of intact males, whereas chronic testosterone administration to female or castrated male animals increased the risk of spontaneous or chemically induced HCC (5–11). These findings raise the possibility that testosterone may promote the development of HCC in humans. In our initial nested case–control study carried out within a cohort of 9691 adult males recruited from six townships of Taiwan, an elevated prediagnostic blood level of testosterone was associated with the risk of HCC after we controlled for confounding by the HBsAg carrier status and other potential HCC risk factors (14). However, in a nested case–control study conducted in a high-incidence area of China, no statistically significant difference in the tertile distribution of blood testosterone between HBsAg-positive male patients with HCC and control subjects was noted, although there was a 50% greater risk for male HBsAg carriers in the highest testosterone tertile relative to those in the lowest testosterone tertile (15). Because these two studies are limited by the small number of case patients studied, a more detailed analysis is required to re-examine the androgen hypothesis involved in the development of HCC.

The action of testosterone is ultimately mediated through the AR, which has been found in HCC and nontumorous liver tissue, but tumor tissues have a higher con-

tent of AR (23). In a rat model for HCC induction, increased hepatic AR expression was observed in female rats during prolonged oral administration of a chemical carcinogen (24). It has also been shown that the growth rate of chemically induced liver tumors in normal male mice is 20-fold higher than the rate in male mice with testicular feminization, which lack functional ARs (11). To our knowledge, this study presents the most direct evidence that AR contributes to the risk of developing HCC in humans by identifying a statistically significant association between the number of AR-CAG repeats and the risk of HCC. The number of AR-CAG repeats is apparently inversely associated with transactivation capabilities of the AR *in vitro* (20–22).

The increased risk of HCC associated with AR genes that have 20 CAG repeats or fewer appears to be relatively modest (OR  $\approx 2$ ). In particular, we observed that the length of the poly(Q) sequence in the AR protein had a statistically significant influence on the risk of developing late-onset HCC only. Furthermore, older male HBV carriers who developed HCC had statistically significantly fewer CAG repeats than the younger patients. Because the grouping of AR-CAG repeats was based on the analysis of the odds of developing HCC, these findings require confirmation. However, fewer AR-CAG repeats (i.e., <19 or 20 repeats) have also been reported to be associated with an increased risk of prostate cancer, which usually occurs in men older than 60 years of age (16–18). A 5%–10% increase in *in vitro* transcriptional activation for each decrement of 10 CAG repeats has been observed (20). Thus, it is reasonable to speculate that length variation within the normal range of repeats, typically 14–35 repeats, observed in this study population cannot have a strong influence on the transcriptional activity *in vivo*. However, subtle differences in AR transactivation activity over a lifetime may have a substantial impact on the transition from the HBV carrier state to HCC.

As we have reported previously (14), men with higher baseline levels of testosterone were more likely to be subsequently diagnosed with HCC than men with lower testosterone levels. It is unlikely that this association can be explained by an effect of prevalent but undiagnosed cancer on plasma testosterone levels. The effect of elevated testosterone levels on the development of HCC was

essentially unchanged when we excluded case patients diagnosed within 4 years of the time blood was drawn. Also, we were not able to explain our results on the basis of confounding. The association with testosterone level remained statistically significant after we incorporated many known HCC risk factors that may be associated with circulatory levels of testosterone into the analysis, such as age, alcohol consumption, chronic liver disease, and cigarette smoking (26,27). Although our study was limited by only a single testosterone measurement for each study subject, testosterone levels in men are relatively stable over time, and thus a single determination of plasma testosterone level should sufficiently represent the long-term hormonal milieu in men. Testosterone levels begin to decline in men at about 40 years of age and decrease roughly 10% per decade thereafter throughout the remainder of life (26). In this study, higher plasma testosterone levels were associated with an increased risk of HCC in younger and older male HBV carriers. However, the association between testosterone level and risk of HCC appears to be stronger in relatively younger men, when levels of this hormone are naturally higher.

The effect of testosterone levels on the risk of HCC may depend on the number of AR-CAG repeats carried by an individual, although we found that the interaction between the two factors was not statistically significant. Male HBV carriers in the highest tertile of testosterone levels and with 20 AR-CAG repeats or fewer had an increased risk of HCC that was approximately fourfold higher than male HBV carriers in the lowest tertile of testosterone levels and with more than 24 AR-CAG repeats (Table 4). This finding is compatible with the results from the mouse model system for testicular feminization, demonstrating that androgens contribute to the development of HCC through AR-mediated mechanisms (11). However, modification of the testosterone effect by the number of AR-CAG repeats may differ between early- and late-onset HCC. Future studies with a substantially larger study population than in this study are required to explore this issue.

In summary, our results suggest that the number of AR-CAG repeats is associated with the risk of HCC among male HBV carriers. There may be an additive effect on HCC risk of elevated testosterone levels in individuals with low num-

bers of AR-CAG repeats, but the exact nature of this relationship remains to be elucidated. Chronic infection with HBV or HCV has been shown to be associated with an increased risk of HCC and account for the vast majority of HCC cases worldwide (1). In Taiwan, HCV seems to play a relatively minor role in the development of HCC. We were not able to examine the androgen hypothesis for HCV-related HCC in the cohort study because of the low prevalence (i.e., <2%) of HCV infection in the general Taiwanese population (1). On the other hand, AR was detected in the liver regardless of sex (23). In light of the animal study indicating increased hepatic AR expression during the development of HCC in female rats (24), it is reasonable to hypothesize that AR may also be involved in the hepatocarcinogenesis among women. Although both male and female HBV carriers have a higher incidence of HCC than noncarriers, the development of HCC occurs with much greater frequency in men than in women (2). We are presently conducting a multicenter case-control study to recruit a sufficient number of female patients with HCC to explore the association of HCC with the number of AR-CAG repeats among women.

## REFERENCES

- (1) Yu MW, Chen CJ. Hepatitis B and C viruses in the development of hepatocellular carcinoma. *Crit Rev Oncol Hematol* 1994;17:71-91.
- (2) Yu MW, Tsai SF, Hsu KH, You SL, Lee SS, Lin TM, et al. Epidemiologic characteristics of malignant neoplasms in Taiwan. II. Liver cancer. *J Natl Public Health Assoc* 1988;8:125-38.
- (3) Yu MW, Gladek-Yarborough A, Chiamprasert S, Santella RM, Liaw YF, Chen CJ. Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. *Gastroenterology* 1995;109:1266-73.
- (4) Yu MW, Chiu YH, Chiang YC, Chen CH, Lee TH, Santella RM, et al. Plasma carotenoids, glutathione S-transferase M1 and T1 genetic polymorphisms, and risk of hepatocellular carcinoma: independent and interactive effects. *Am J Epidemiol* 1999;149:621-9.
- (5) Agnew LR, Gardner WU. The incidence of spontaneous hepatomas in C3H, C3H (low milk factor), and CBA mice and the effect of estrogen and androgen on the occurrence of these tumors in C3H mice. *Cancer Res* 1952;12:757-61.
- (6) Vesselinovitch SD, Mihailovich N. The effect of gonadectomy on the development of hepatomas induced by urethan. *Cancer Res* 1967;27:1788-91.
- (7) Toh YC. Effect of neonatal castration on liver tumor induction by N-2-fluorenylacetamide in suckling BALB/c mice. *Carcinogenesis* 1981;2:1219-21.
- (8) Firminger HI, Reuber MD. Influence of adrenocortical, androgenic, and anabolic hormones on the development of carcinoma and cirrhosis of the liver in AXC rats fed N-2-fluorenyldiacetamide. *J Natl Cancer Inst* 1961;27:559-95.
- (9) Reuber MD. Influence of hormones on N-2-fluorenyldiacetamide-induced hyperplastic hepatic nodules in rats. *J Natl Cancer Inst* 1969;43:445-52.
- (10) Vesselinovitch SD, Itze L, Mihailovich N, Rao KV. Modifying role of partial hepatectomy and gonadectomy in ethylnitrosourea-induced hepatocarcinogenesis. *Cancer Res* 1980;40:1538-42.
- (11) Kemp CJ, Leary CN, Drinkwater NR. Promotion of murine hepatocarcinogenesis by testosterone is androgen receptor-dependent but not cell autonomous. *Proc Natl Acad Sci U S A* 1989;86:7505-9.
- (12) Mokrohisky ST, Ambruso DR, Hathaway WE. Fulminant hepatic neoplasia after androgen therapy [letter]. *N Engl J Med* 1977;296:1411-2.
- (13) Farrell GC, Joshua DE, Uren RF, Baird PJ, Perkins KW, Kronenberg H. Androgen-induced hepatoma. *Lancet* 1975;1:430-2.
- (14) Yu MW, Chen CJ. Elevated serum testosterone levels and risk of hepatocellular carcinoma. *Cancer Res* 1993;53:790-4.
- (15) Yuan JM, Ross RK, Stanczyk FZ, Govindarajan S, Gao YT, Henderson BE, et al. A cohort study of serum testosterone and hepatocellular carcinoma in Shanghai, China. *Int J Cancer* 1995;63:491-3.
- (16) Ross RK, Pike MC, Coetzee GA, Reichardt JK, Yu MC, Feigelson H, et al. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. *Cancer Res* 1998;58:4497-504.
- (17) Ingles SA, Ross RK, Yu MC, Irvine RA, La Pera G, Haile RW, et al. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. *J Natl Cancer Inst* 1997;89:166-70.
- (18) Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Brufsky A, Talcott J, et al. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. *Proc Natl Acad Sci U S A* 1997;94:3320-3.
- (19) Rebbeck TR, Kantoff PW, Krithivas K, Neuhausen S, Blackwood MA, Godwin AK, et al. Modification of BRCA1-associated breast cancer risk by the polymorphic androgen-receptor CAG repeat. *Am J Hum Genet* 1999;64:1371-7.
- (20) Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res* 1994;22:3181-6.
- (21) Kazemi-Esfarjani P, Trifiro MA, Pinsky L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG)<sub>n</sub>-expanded neuronopathies. *Hum Mol Genet* 1995;4:523-7.
- (22) Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA. Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. *Hum Mol Genet* 2000;9:267-74.
- (23) Nagasue N, Ito A, Yukaya H, Ogawa Y. Androgen receptors in hepatocellular carcinoma and surrounding parenchyma. *Gastroenterology* 1985;89:643-7.
- (24) Ostrowski JL, Ingleton PM, Underwood JC, Parsons MA. Increased hepatic androgen receptor expression in female rats during diethylnitrosamine liver carcinogenesis: a possible correlation with liver tumor development. *Gastroenterology* 1988;94:1193-200.
- (25) Chen CJ, Yu MW, Liaw YF, Wang LW, Chiampasert S, Matin F, et al. Chronic hepatitis B carriers with null genotypes of glutathione S-transferase M1 and T1 polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma. *Am J Hum Genet* 1996;59:128-34.
- (26) Dai WS, Kuller LH, LaPorte RE, Gutai JP, Falvo-Gerard L, Caggiola A. The epidemiology of plasma testosterone levels in middle-aged men. *Am J Epidemiol* 1981;114:804-16.
- (27) Johnson PJ. Sex hormones and the liver. *Clin Sci (Colch)* 1984;66:369-76.
- (28) Yu MW, Chang HC, Liaw YF, Lin SM, Lee SD, Liu CJ, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 2000;92:1159-64.
- (29) Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, et al. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet* 1992;339:943-6.
- (30) Dunsford HA, Sell S, Chisari FV. Hepatocarcinogenesis due to chronic liver cell injury in hepatitis B virus transgenic mice. *Cancer Res* 1990;50:3400-7.
- (31) Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991;351:317-20.

## NOTES

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