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Viral Factors and Outcomes of Chronic HBV Infection

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Abstract: Viral factors associated with outcome of chronic hepatitis B virus (HBV) infection include hepatitis B e antigen status, HBV DNA, genotype, and HBV variants. Mutations in the HBV core promoter region have been shown to be independently associated with hepatocellular carcinoma (HCC). The most common core promoter mutations involve a double substitution A1762T and G1764A (TA). Besides TA mutations, several other core promoter changes have been reported to be associated with the development of cirrhosis and HCC. Future studies should determine if detection of these changes can predict the outcome of patients with chronic HBV infection.

Am J Gastroenterol 2011; 106:93–95; doi:10.1038/ajg.2010.404

Worldwide, chronic hepatitis B virus (HBV) infection accounts for more than 500,000 deaths each year. Most of the deaths are a result of complications of cirrhosis although hepatocellular carcinoma (HCC) can also occur in patients who do not have cirrhosis. Viral, host, and environmental factors have been reported to be associated with the risk of cirrhosis and HCC.

Viral factors associated with outcome of chronic HBV infection include hepatitis B e antigen (HBeAg) status, HBV DNA, HBV genotype, and HBV variants. A study of 2,361 hepatitis B surface antigen (HBsAg)-positive men (age 30–65 years) in Taiwan followed for 92,359 person-years found that those who were HBeAg positive at enrollment had sixfold higher risk of HCC than those who were HBeAg negative (1). Another study followed 483 HBeAg-positive patients (mean age 29 years) for 11.7 years showed that delayed HBeAg seroconversion (after age 40) was associated with a hazard ratio (HR) of 5.22 for HCC compared with those who seroconverted before age 30 (2).

Several large cohort studies have shown that high serum HBV DNA is associated with increased risk of cirrhosis, liver-related death, and HCC. The best-characterized study, REVEAL study, followed 3,653 HBsAg carriers (age 30–65 years) recruited from seven communities in Taiwan for a mean of 11.4 years. Subjects with HBV DNA >4 log10 copies per ml had 8-, 11-, and 11-fold risk of cirrhosis, liver-related death, and HCC, respectively, compared with those with lower HBV DNA levels (3–5).

HBV genotype is also reported to be associated with cirrhosis and HCC development. On the basis of an intergroup divergence of 8% or more in the complete nucleotide sequences, 10 HBV genotypes have been identified. The prevalence of various HBV genotypes varies geographically; genotypes B and C account for more than 90% of chronic HBV infection in East Asia. Numerous studies have reported that HBV genotype C is independently associated with a higher risk of HCC than genotype B (6). Genotype C is also reported to be associated with more rapid progression to cirrhosis than genotype B (7). Data on HBV genotypes other than B and C and HCC are limited. Studies outside Asia have reported an association between HCC and genotype F in Alaska (8), and genotype Aa in South Africa (9).

Because of the high error rate with viral reverse transcriptase, spontaneous mutations in the HBV genome often emerge during the course of chronic HBV infection. Mutations in the precore and core promoter regions are the most common. They abolish or downregulate HBeAg production and are frequently found in patients with HBeAg-negative chronic hepatitis. Mutations in the core promoter region have also been found to be associated with increased risk of HCC (10). The HBV core promoter region includes the basal core promoter and the enhancer II. The core promoter region regulates transcription of precore messenger RNA (and HBeAg production) and pregenomic RNA (HBV replication and hepatitis B core antigen (HBcAg) expression). It also overlaps with the HBx gene, which has been incriminated in hepatocarcinogenesis (11).

The most common core promoter mutation involves a double substitution A1762T and G1764A (TA) in the basal core promoter region. A cross-sectional study of 694 patients with chronic HBV infection in the United States found that the TA mutation was present in 36% of HBeAg-positive and in 51% of HBeAg-negative patients (12). Some studies reported that the TA mutation is associated with higher aminotransferases and higher inflammation scores on liver biopsies (13). *In vitro* studies suggest that core promoter mutations increase the rate

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Received 28 June 2010; accepted 3 September 2010

of HBV replication but clinical data correlating core promoter mutations and serum HBV DNA levels are conflicting (14). Many studies, mostly from Asian countries, have reported an association between the TA mutation and HCC (15). The TA mutation is more commonly found in patients with genotype C than those with genotype B; however, an independent association between the TA mutation and risk of HCC was clearly shown in the REVEAL study (6). After adjustment for serum HBV DNA level and HBV genotype, the HR and 95% confidence interval (95% CI) of the TA mutation for HCC was 1.73 (95% CI 1.13–2.67); by contrast, the HR for the most common precore stop codon mutation (G1896A) was 0.34 (95% CI 0.21–0.57). Besides TA mutations, other core promoter mutations have also been reported to be associated with an increased risk of HCC (16). These other mutations were almost invariably found in association with the TA mutation, suggesting a key role for the TA mutation in hepatocarcinogenesis. Longitudinal studies have shown that the TA mutation is selected earlier—many years before HCC diagnosis—whereas the other core promoter mutations emerge later, closer to the time of HCC diagnosis (17). These clinical data suggest that the TA mutation is important in the early stages of carcinogenesis but additional mutations may be necessary for HCC development. Therefore, a key question is which other mutations are critical in HCC development and whether the presence of these mutations can be used to predict the risk of HCC.

In this issue of the Journal, Yin *et al.* sequenced the HBV core promoter region in serum samples from 846 asymptomatic carriers (ASC), 235 patients with chronic hepatitis, 188 patients with cirrhosis, and 190 patients with HCC (18). Roughly three-quarters (72%) of the patients had genotype C infection. Stepwise multivariate regression analysis found that among the patients with genotype C infection, age, gender, abnormal alanine aminotransferase (ALT), and core promoter mutations: T1768A, A1762T/G1764A (TA) and A1846T, were independently associated with cirrhosis. Independent factors associated with HCC included age, abnormal ALT, HBV DNA ≥4 log10 copies per ml, genotype C, and core promoter mutations: C1653T, T1674C/G, T1753V, and A1762T/G1764A (TA). Apart from the T1674C/G mutation, these findings are in agreement with previous studies (16). A previous meta-analysis found that the frequency of C1653T, T1753V, and TA mutations increased successively from ASC to cirrhosis (16). This study confirmed that even after adjustment for age (and duration of chronic HBV infection), core promoter mutations are associated with HCC.

The major strength of this study is the large sample size. Whereas other studies have focused on HCC, the authors also identified core promoter mutations that were associated with cirrhosis. However, there are some weaknesses in this study. First, the criteria for classifying the patients as ASC, chronic hepatitis, cirrhosis, or HCC were not standard. For example, the criteria for ASC required only one normal ALT regardless of HBeAg or HBV DNA status. Second, this was a cross-sectional study. The sequence in which the core promoter mutations emerge and the interval between the emergence of these mutations and the diagnosis of cirrhosis or HCC cannot be determined; therefore, the utility of the core promoter mutations found in this study to identify a subset of HBV carriers who are at greater risk of cirrhosis or HCC cannot be assessed. Third, data in Table 2 suggest that mutations associated with cirrhosis and HCC development are different. At positions 1673, 1726, 1727, 1730, 1768, 1773, and 1779, the sequence in patients with HCC is closer to that in patients with chronic hepatitis than those with cirrhosis, yet the authors indicated that 73% of the patients with HCC had cirrhosis. Although mutations can be reversed, the likelihood that mutations at so many positions were selected as the disease progresses to cirrhosis only to revert back to wild-type sequence when HCC develops is very low. Stratification of the HCC patients into those with and without cirrhosis would clarify if the latter patients were the ones whose sequences at those positions were wild type and not mutations associated with cirrhosis.

The paper by Yin *et al.* provided additional evidence on the importance of core promoter mutations and progression of HBV-related liver disease. Future studies should focus on the utility of these mutations in predicting outcomes and the mechanisms by which core promoter mutations increase the risk of cirrhosis or HCC.

CONFLICT OF INTEREST

YueHua Huang performed the literature search and wrote the draft, Anna S.F. Lok provided guidance and reviewed and revised the article. Dr Huang was supported by the Ravitz Foundation, the CK Hui Memorial Fund, and the Tuktawa Foundation. There is no competing interest.

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