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# Hepatitis B Virus Core Variants, Liver Fibrosis, and Hepatocellular Carcinoma

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1 Chronic hepatitis B virus (HBV) infection is a major cause of advanced liver  
2 disease and hepatocellular carcinoma (HCC) world-wide (1). Current antiviral treatments  
3 control HBV replication and reduce progressive liver disease. However, viral cure is  
4 rarely achieved and patients still remain at risk for HCC (1). The pathogenesis of HBV-  
5 induced HCC is thought to be multifactorial with both direct and indirect mechanisms (2):  
6 HBV-related HCCs can also arise in non-cirrhotic livers, supporting the notion that HBV  
7 plays a direct role in liver transformation by triggering both common and etiology specific  
8 oncogenic pathways in addition to stimulating the host immune response and driving  
9 liver chronic necro-inflammation. Moreover, certain viral genotypes and HBV variants  
10 have been proposed to increase HCC risk (3): Epidemiological studies suggest that HBV  
11 genotypes C, D and F carry a higher lifetime risk of HCC development than genotype A  
12 and B (1). In the United States, Alaska Native (AN) individuals are characterized by the  
13 highest HBV seroprevalence, with a high incidence of HCC. HBV genotype F and its  
14 subtype 1 (F1) is highly prevalent in AN individuals with HCC and has therefore  
15 suggested to predispose to HCC (4,5). Among naturally occurring viral variants, HBV  
16 core promoter and precore/core mutants have been shown to be associated with  
17 enhanced HCC risk (6). Mutations in the basal core promoter (BCP) region enhance  
18 viral replication by creating a binding site for hepatocyte nuclear factor (HNF) protein  
19 leading to increased transcription of viral RNA (7) or enhanced viral encapsidation (8).  
20 The double mutation A1762T/G1764A is the most common mutation found in the BCP.  
21 In the pre-core region (PC), the predominant mutation is G1896A, which modulates of  
22 HBeAg synthesis at the translational level by introducing a stop codon (6). However,  
23 there are only few functional studies investigating the functional relationship between  
24 genetic alterations and disease biology in *in vivo* model systems. A better understanding  
25 of the functional relationship between HBV variants and their impact on liver disease  
26 and carcinogenesis is important not only for the understanding of their mechanism of  
27 action but also has potential impact for determining HCC risk and patient management.

28 In this issue of *Hepatology*, a collaborative research effort between the teams of  
29 Dr. Brian J. McMahon, Anchorage and Prof. Yasuhito Tanaka, Nagoya, Japan has  
30 addressed this important question by identifying genetic variants associated with HCC  
31 and investigating their phenotype in cell culture and animal models (9). Hayashi and

1 colleagues first analyzed the clinical and virological significance of genotype F1b in long-  
2 term serial samples from 20 HCC patients with HBV infection. The authors showed that  
3 all isolates were of genotype F1b. Core T1938C (V13A) and A2051C (N51H) mutations  
4 had accumulated together with BCP and PC mutations, suggesting a functional  
5 association between these variants and hepatocarcinogenesis (9). The N-terminal part  
6 of core protein is crucial for self-assembly, while the C-terminal part is essential for  
7 nucleocapsid formation. Interestingly, T1938C and A2051C mutations are located in the  
8 capsid assembly domain of the core protein. Using an expression cloning approach  
9 combined with site-directed mutagenesis, the authors observed that the core A2051C  
10 mutation enhances HBV replication by stabilizing core protein dimerization putatively  
11 facilitating nucleocapsid formation. Next, to address the structure-function relevance of  
12 the variants selected in HCC *in vivo* they used human liver chimeric mice - a state-of-  
13 the-art animal model for the study of HBV infection in engrafted human hepatocytes in  
14 the mouse liver. Chimeric mice were inoculated with wild-type virus or recombinant virus  
15 containing the BCP A1762T/G1764A mutations, the PC G1896A mutation or the  
16 combination BCP/PC/2051. Interestingly, the variants exhibited higher levels of  
17 replication and protein expression than the wild-type strain as indicated by enhanced  
18 levels of HBV DNA and HBsAg in the serum of infected mice.

19 To better understand the disease biology associated with the mutations, the  
20 authors investigated the functional consequences of viral infection on the liver  
21 transcriptome using microarray. Comparative analyses between wild-type and mutant  
22 strains showed that the mutations resulted in the upregulation of the expression of  
23 genes associated with cell proliferation and hepatocarcinogenesis: these included MYC  
24 (c-myc avian myelocytomatosis viral oncogene homolog), GAB2 (Grb2-Associated  
25 Binding Protein 2), BDKRB2 (bradykinin receptor B2), FST (follistatin) and MAP3K8  
26 (mitogen-activated protein). Furthermore, the authors observed that the BCP/PC/2051  
27 mutations were associated with a modulated expression of liver inflammation and  
28 fibrosis-related genes such as C-X-C chemokine receptor type 7, endothelin 1, insulin-  
29 like growth factor binding protein 3 and intercellular adhesion molecule 1 (Figure 1).

30 Finally, the authors studied the liver disease biology associated with this variant  
31 using detailed histopathological analyses of the humanized part of the livers.

1 Importantly, infections with the core variant appeared to be associated with fibrosis in  
2 the liver of HBV-infected chimeric mice. The authors also observed an enhanced  
3 inflammation, although the meaning of this finding remains to be determined since the  
4 immune system of these mice is heavily disturbed by the severe combined  
5 immunodeficiency (SCID) phenotype. Additional *in vivo* studies using recombinant  
6 viruses containing BCP/PC mutations with and without the A2051C or the T1938C  
7 mutation revealed that the phenotype of fibrosis appeared to be predominantly caused  
8 by the A2051C mutation.

9 Collectively, these data provide strong evidence that this variant may have a  
10 causal role for HCC observed in the AN patients. One potential limitation of the study is  
11 that the variant infection did not result in HCC in the mouse model. However, the  
12 absence of hepatocarcinogenesis is a known feature of this animal model, which is most  
13 likely due the absence of a functional immune system with lack of inflammatory pro-  
14 carcinogenic antiviral immune responses. Alternatively, the duration of the experiment  
15 was not long enough to observe hepatocarcinogenesis. Collectively, this study provides  
16 an important advancement in the field by showing convincing evidence of a robust  
17 structural and functional relationship between a patient-derived core variant and liver  
18 disease biology. In particular, the observation of variant-associated liver fibrosis is an  
19 important finding since fibrosis stage has been shown to be a key predictor and driver of  
20 hepatocarcinogenesis in various etiologies (10).

21 What are the clinical implications of these findings and the next studies further  
22 advancing the findings of the authors? First, it would be of interest to study the  
23 prevalence of the identified core mutation in a larger Alaskan cohort as well as cohorts  
24 infected with other HBV genotypes. Further studies may also address the question  
25 whether certain genotypes, such as genotype F, predispose to the mutation by  
26 genotype-specific sequences. Finally, it would be of interest to study whether or not the  
27 mutants are associated with human HCC that occurs in the absence of liver cirrhosis.  
28 These studies would give important insights of the impact and prevalence of the  
29 mutation the association with HCC risk in general. It may be of interest to assess  
30 whether the detection of this variant could serve as an additional predictor of HCC risk  
31 triggering e.g. more intense surveillance of patients harboring this mutation. Second, a

1 follow-up study on the molecular virus-host interactions of this variant may potentially  
2 provide an opportunity to uncover novel targets for chemopreventive approaches for  
3 HCC.

4 By providing a convincing structure-function relationship of the core mutant with  
5 liver fibrosis in a state-of-the-art animal model, the study significantly advances our  
6 understanding of the role of variants in the pathogenesis of liver disease and HCC and  
7 will stimulate further research on the role of viral factors in hepatocarcinogenesis.

8  
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14  
15 **Conflict of interest.** The authors declare no conflict of interest.

16  
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- 13

1 **Figure legend**

2 **Figure 1: HBV core mutations associated with fibrosis and hepatocarcinogenesis.**

3 **A.** Schematic representation of the HBV genome with clinical mutations. An  
4 accumulation of Core T1938C and A2051C mutations together with BCP  
5 (A1762T/G1764A) and PC (G1896A) mutations were observed in Alaskan native HCC  
6 patients (9). The mutations, leading to an increased viral replication, are indicated by  
7 stars on the genome. **B.** Effects of HBV core mutants on the HBV life cycle and host cell  
8 pathways in liver chimeric mice as observed by Hayashi et al. (9). Following viral entry  
9 mediated by HSPG and NTCP, the HBV genome is released into the nucleus where  
10 rcDNA is converted into cccDNA. cccDNA serves as template for viral transcripts  
11 including pgRNA, which is encapsidated and reverse transcribed into de novo HBV  
12 genomic DNA before assembly and release of the viral particle. Hayashi *et al.*  
13 demonstrated that core mutations enhance HBV replication in genotype F1b. In human  
14 liver chimeric mice infection with mutant virus resulted in up-regulation/activation of  
15 pathways potentially driving fibrosis and carcinogenesis. Abbreviations: BCP, basal core  
16 promotor; c-MYC, c-myc avian myelocytomatosis viral oncogene homolog; cccDNA,  
17 covalently closed circular DNA; CXCR7, C-X-C chemokine receptor type 7; FGF9,  
18 fibroblast growth factor 9; FGFR, fibroblast growth factor receptor; ICAM-1, intercellular  
19 adhesion molecule 1; MAP3K8, mitogen-activated protein; NTCP, NA<sup>+</sup>-taurocholate  
20 co-transporting polypeptide; P, polymerase; HSPG, heparan sulfate proteoglycan; PC,  
21 core promotor; pgRNA, pregenome RNA; PreS, PreS region; S, surface protein; X, X  
22 protein.

