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# Core Promoter Mutation of ntC1731T and G1806A of Hepatitis B Virus Increases HBV Gene Expression

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Chronic infection by hepatitis B virus (HBV) greatly increases the risk for liver cirrhosis and hepatocellular carcinoma (HCC). The outcome of HBV infection is shaped by the complex interplay of the mode of transmission, host genetic factors, viral genotype, adaptive mutations, and environmental factors. The pregenomic RNA transcription of HBV for their replication is regulated by the core promoter activation. Core promoter mutations have been the reason for acute liver failure and are associated with HCC development. We obtained HBV genes from a patient in Myanmar who was infected with HBV and identified gene variations in the core promoter region. For measuring the relative transactivation activity of the core promoter, we prepared the core-promoter reporter construct. Among the gene variations of the core promoter, the mutations of C1731T and G1806A were associated with increase in the transactivation of the HBV core promoter. Through computer analysis for searching for a tentative transcription factor binding site, we showed that the mutations of C1713T and G1806A newly created C/EBPβ and XBP1-responsive elements of the core promoter, respectively. The ectopic expression of C/EBPβ largely increased the HBV core promoter containing the C1713T mutation and that of XBP1 activated the M95 promoter containing the G1806A mutation. Our efforts to treat and prevent HBV infections are hampered by the emergence of drug-resistant mutations and vaccine-escape mutations. Our results provide the biological properties and clinical significance of specific HBV core promoter mutations.

**Key words** : C/EBPβ, core promoter, HBV, virus mutation, XBP1

## Introduction

Hepatitis B virus (HBV) infection causes various liver diseases including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [9]. HBV was infected in almost one-third of the world's population are infected with HBV and about 350 million people are chronically infected [13]. Based on differential geographical HBV infection pattern, in the United States and Europe, HBV prevalence is low at 0.1~0.2% whereas, in the Mediterranean coast and Japan HBV prevalence is about 2.0~7.0%. In China, Southeast Asia, and Sub-Saharan Africa, HBV prevalence is high and ranges from 8.0% to 20.0% [14]. Hepatitis B is one of the most common and serious disease in Myanmar. Therefore, this research aims to further the development of more effective treatments that are specific to mutations and genotype. HBV can be classified into ten genotypes, A through J, based on a sequence divergence of > 8%. Within the viral genotypes are sub genotypes that differ above 4%. The mutation rate of HBV is higher than that of other DNA viruses owing to the reverse transcription in its life cycle.

The core promoter in the viral genome plays an important role in HBV replication [5, 24], and is found in front of the start site of the genomic RNA [4]. Covalent closed circular DNA (cccDNA) of HBV is used as a template for the transcription of mRNA [2, 10]. HBV-transcribed mRNA comprises a 3.5 kb pre-Core (pre-C) mRNA, a 3.5 kb Pre-genomic (pg) RNA, a 2.4 kb large (L), a 2.1 kb middle (M), small (S) mRNA, and a 0.7 kb X mRNA. Pre-C mRNA is translated into pre-C protein, which produces serological hepatitis B e antigen (HBeAg). L, M, and S mRNA are translated to produce envelope proteins and HBx mRNA is translated to HBx protein. Pre-genomic RNA is little shorter than pre-core mRNA. The two AUG start codons for the transcription of pre-genomic RNA and pre-core mRNA are present in same

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reading frame [27].

Although HBeAg is not a necessary component for HBV virion formation, it is counted as an important marker for measuring the level of HBV replication. Low levels of HBeAg and high levels of Anti-HBe are associated with low levels of HBV replication [6]. A high mutation rate during HBV replication results in resistance to antiviral therapies [16]. Chronic HBV showing HBeAg-negative was seen in 24% of patients when HBeAg seroconversion was observed for 8.5 years [15]. Moreover, HBeAg-negative chronic HBV reappeared in the period of HBV retention [7]. Precore mutation (G1896A) or core promoter mutation (A1762T and G1764A) was detected in most HBeAg-negative chronic hepatitis B patients [17]. Pre-core and core promoter mutations resulted in the inhibition of HBeAg synthesis. The report suggested that these mutations are associated with clinical reactivation; however, precore mutations are also detected during the non-proliferative period. It is unclear whether these mutations directly induce inflammation of the liver [3, 20]. This study indicates that the sporadic core promoter mutations of HBV increases HBV gene expression, resulting from new creation of liver-specific transcription factor binding sites for HBV DNA replication.

## Materials and Methods

### Serum samples

This study used serum samples of 96 HBsAg positive patients that were received for routine HBV DNA detection from Department Medical Research (DMR) in Yangon Myanmar. The serum samples were stored at -80℃. This study was approved by Ethics Review Committee of Department of Medical Research. The approval number is 22/ Ethics 2015, dated 25.3.2015. Informed consents were obtained from the study participants who were at or more than 16 years and from the parents/guardians of the study participants who were under 16 years.

### Amplification of viral DNA by the PCR

HBV DNA was extracted from 96 serum of HBV HBsAg positive patients. The 40 µl of HBsAg positive serum was diluted 4-fold in PBS. The diluted samples were incubated for 3 min at 95℃ and for 5min at 4℃. After centrifugation is used at 12,000 rpm for 20 min, the supernatants 100 µl transfer to 1.5 ml microcentrifuge tubes. Collected supernatant is used to DNA template for PCR. HBV core promoter and preS region was amplified using specific gene primers. PCR of HBV core promoter region was performed using nTaq DNA polymerase (Enzynomics, Daejeon, Republic of KOREA) and primary and nested primer. Amplified PCR products of HBV DNA were examined by electrophoresis on a 1% agarose gel at 150 V for 25 min and stained with ethidium bromide.

### Sequencing and HBV mutation analysis

The purified PCR products of HBV DNA sent to a DNA sequencing company (COSMO GENETECH, Daejeon, Korea) for sequencing. HBV core promoter mutation site were analyzed using the BioEdit and multiple sequence alignment service at CLUSTALW home page (http://www.genome.jp/tools/clustalw/) in the HBV nucleotide sequences.

### Plasmid constructs and reagents

To construct HBV core promoter M50, M82, M83 and M95, the promoter fragments from serum of HBV infected patients were amplified by PCR using cloning primers contacting restriction enzyme site, KpnI and HindIII: forward, 5′-GCC GGT ACC ACG CCC ACC AAA TAT - 3′ and reverse, 5′-CGC AAG CTT TAC AAG AGA TGA TTA-3′. The PCR products were digested with KpnI and HindIII were cloned into pGL3B vector. The sequences were confirmed by COSMO Gene Tech. The transfection reagents Polyfect and jetPEI were purchased from QIAGEN (Hilden, Germany) and Polyplus Transfection (Illkirch, France).

### Cell culture

Chang liver cells (all obtained from the American Type Culture collection, Manassas, VA, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO BRL, Grand Island, NY, USA) and 1% (v/v) penicillin-streptomycin (GIBCO BRL) at 37℃ in a humid atmosphere containing 5% $CO_2$.

### Luciferase assay

Chang liver cells were seeded in a 24-well culture plate and transfected with reporter vector and β-galactosidase expression plasmid, along with each indicated expression plasmids using PolyFect (QIAGEN). The pcDNA3 plasmid DNA was added to accomplish the same amount of plasmid DNA transfection. After 24 hr of transfection, the cells were washed with ice-cold PBS and lysed with the cell culture lysis buffer (Promega, Madison, WI, USA). Luciferase activity was

determined using an analytical luminescence luminometer according to the manufacturer's instructions. Luciferase activity was normalized for transfection efficiency using the corresponding β-galactosidase activity. All assays were conducted at least in triplicate.

### Transcription factor search

Blast searches of GenBank were performed using the BLAST service at the National Center for Biotechnology Information (NCBI) home page (http://www.ncbi.nlm.nih.gov/). For analyzing transcription factor binding sites in the HBV core promoter region, the PROMO3 service was used.

### Statistical analysis

Statistical software GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA, USA) was used for analysis. All experiments are presented as mean ± standard deviation (SD) and analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. *p*-value of <0.05 was considered statistically significant.

## Results

### Specific core promoter mutation sites are found in HBV genes obtained from Myanmar hepatocellular carcinoma patients

HBV contain four overlapping open reading frames for HBV replication including core promoter for synthesis of pre-genomic RNA [4]. Mutations in the core promoter result in dysregulated HBV replication. An increased viral load by core promoter mutation in the HBV-infected liver cells triggers liver pathogenesis. We have collaborated Myanmar researchers, who are members of Department of Medical Research of Myanmar, for analyzing HBV gene variation from HBV-infected patients in Myanmar. Myanmar has high frequency of liver diseases, such as hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), resulting from HBV infection. We presented HBV core promoter sequence which was found in non-liver disease case (HBV M50) (Fig. 1).

After obtaining HBV genes of Myanmar patients, we analyzed gene sequences of core promoter. HBV core promoter M50, M82, M83, and M95 constructs contain the extracted core promoter DNA from the serum of Myanmar patients 50, 82, 83, and 95 with HCC. To identify mutations in the core promoter, serum from the core promoter site was amplified using PCR. Sequencing and alignment reactions were conducted on the PCR products. Mutations in the core promoter DNA were found at nt1713 and nt1798 of core promoter M82, at nt1713 only of core promoter M83 and nt1806 of the core promoter M95 (Fig. 2A).

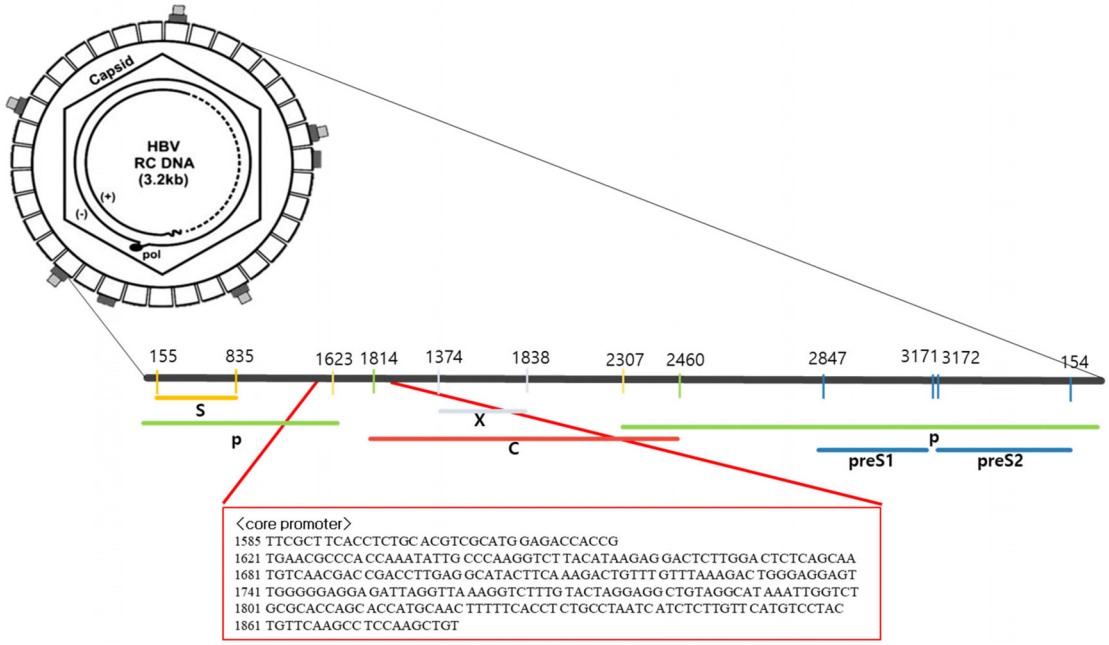


Fig. 1. Identification of DNA sequence of HBV core promoter from M50. After obtaining HBV gene from M50 patient, the core promoter region (nt1595 – nt1880) was sequenced. The M50 core promoter sequence was used as a wild type and control for comparing with other the mutated core promoter sequences.

### C1713T and G1806A mutations increase the transactivation of HBV core promoter

In order to analyzing the core promoter activity quantitatively, we constructed the luciferase reporter system containing HBV core promoter (Fig. 2B). Based on the luciferase reporter construct, we constructed each four luciferase reporter plasmids conjugating with core promoter sequences of M50, M82, M83, and M95. We transfected with HBV core promoter M50, M82, M83, and M95 constructs into Chang liver cells and measured the luciferase activity for evaluating the relative promoter activity of each core promoter variation. The transactivation activity of core promoters of M82, M83 and M95 showed much stronger than M50 promoter activation in Chang liver cells (Fig. 2C). This result indicated that HCC-related gene variation in the core promoter increases the transactivation of HBV core promoter.

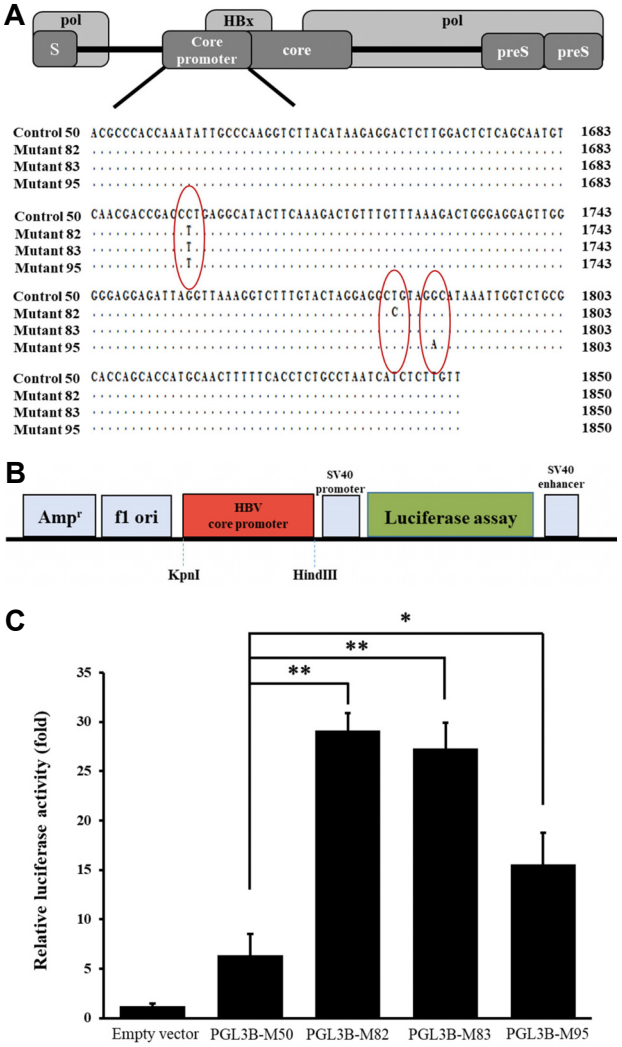


Fig. 2. HBV core promoter mutation induces HBV gene expression. (A) The HBV core promoter site from the patients was sequenced and the sequences were aligned to enable the detection of mutations. (B) Structure of the HBV core promoter reporter plasmid. HBV core promoter region from the serum of Myanmar patients M50, M82, M83, and M95 was amplified by PCR and was cloned using a PGL3B vector. (C) The effect of mutations on HBV core promoter activity. Chang liver cell were transfected with HBV core promoter M50, M82, M83, and M95 reporter construct for 24 hr. The cell lysates were determined for luciferase activity. Cloned M50 served as the control. The bar represents the mean value from at least three independent experiments. *$p$<0.05, **$p$<0.01 indicate significance when compared to C/EBPβ transfectant.

### Transcription factor C/EBPβ increases C1713T-containing core promoter transactivation

For the pre-genomic transcription of HBV, liver cell-specific transcription factors should bind to the core promoter and induce the core promoter activation. We searched the tentative binding sites of specific transcription factors in the M50 control and three point mutated promoters (M82, M83, and M95) using PROMO program. The results show that RXRα, GRα, EBF bind to the HBV core promoter control M50 at nt1713. Transcription factors GRα, C/EBPβ bind to the mutation site nt1713 of HBV core promoter M82, M83, and M95 (Fig. 3A). C/EBPβ binds to the HBV core promoter and regulates HBV gene expression [12, 23]. There was not found for any transcription factor to bind to the mutation site nt1798 of HBV core promoter M82.

To examine whether C/EBPβ is responsible to increase the core promoter activation of M82, M83, and M95, we tried to transfect each reporter plasmids (M50-luc, M82-luc, M83-luc, and M95-luc) into Chang liver cells in the presence or absence of C/EBPβ cotransfection and measured the luciferase activity for evaluating relative transactivation of core promoter variation. As shown in Fig. 3B, C/EBPβ largely increased the core promoter activity of M82, M83, and M95 compared to M50 promoter activity. This result indicated that the enriched liver transcription factor C/EBPβ might respond well to the core promoter including C1713T gene variation and increase production of pre-genomic RNA for upregulation of HBV replication.

### Transcription factor XBP1 increases G1806A-containing core promoter transactivation

Transcription factors XBP1, AP-1α and GRα bind to HBV core promoter mutant M95 at nt1806 (Fig. 4A). Comparison of transcription factors to bind to between M50 and M95

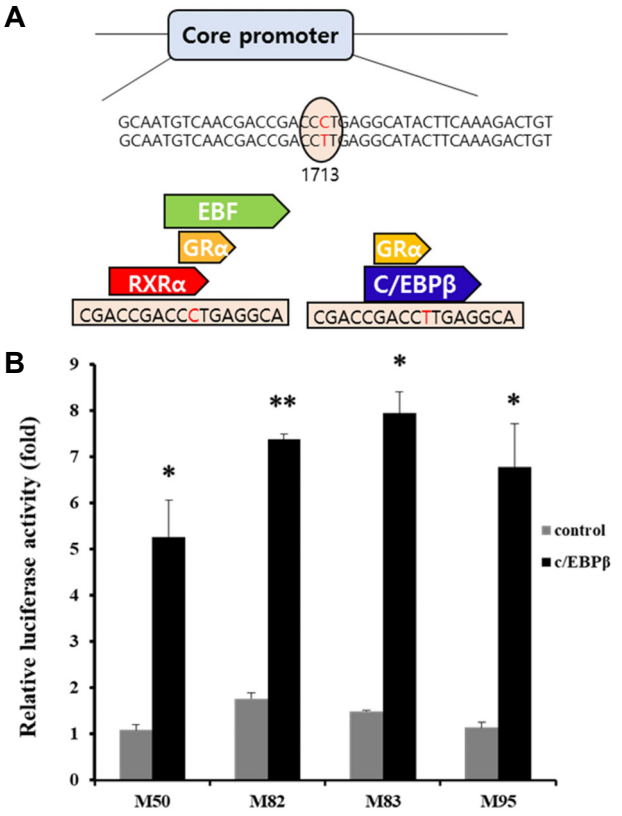


Fig. 3. C/EBPβ is responsible to increase HBV core promoter activity of C1713T mutation. (A) Creation of C/EBPβ binding site on the C1713T core promoter of M82, M83, and M95. The transcription factor binding sites to the HBV core promoter mutation sites at nt1713 was searched through PROMO (http://alggenlsi.upc.es/cgibin/promo_V3/promo/Promoinit.cgi?-dirDB=TF_8.3). (B) The effect of C/EBPβ binding on HBV core promoter activity. Chang live cells were transfected with HBV core promoter M50, M82, M83, and M95 construct for 24 hr in the presence or absence of C/EBPβ transfection. The cell lysates were applied for luciferase activity. Cloned M50 served as the control. The bar represents the mean value from at least three independent experiments. *$p$<0.05, **$p$<0.01 indicate significance when compared to C/EBPβ transfectant.

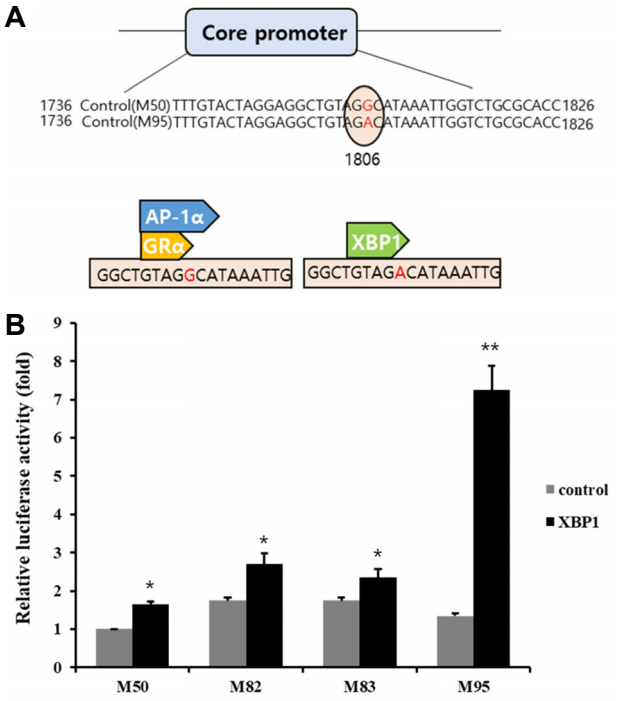


Fig. 4. XBP1 is responsible to increase HBV core promoter activity of G1806A mutation. (A) Creation of XBP1 binding site on the G1806A core promoter of M82, M83, and M95. The transcription factor binding sites to the HBV core promoter mutation sites at nt1806 was searched through PROMO (http://alggenlsi.upc.es/cgibin/promo_V3/promo/Promoinit.cgi?-dirDB=TF_8.3). (B) The effect of XBP1 binding on HBV core promoter activity. Chang live cells were transfected with HBV core promoter M50, M82, M83, and M95 construct for 24 hr in the presence or absence of XBP1 transfection. The cell lysates were applied for luciferase activity. Cloned M50 served as the control. The bar represents the mean value from at least three independent experiments. *$p$<0.05, **$p$<0.01 indicate significance when compared to XBP1 transfectant.

showed that XBP1 binding site was exclusively found at M95 core promoter including G1806A gene variation. In order to confirm whether M95 core promoter is responsible to XBP1, we applied the transient transfection of each core promoter constructs in the presence or absence of XBP1 transfection. XBP1 overexpression significantly increased M95 core promoter, but not other promoters (Fig. 4B). XBP1 is responsible to transmit endoplasmic reticulum (ER) stress response. HBV infection and HBV protein expression are known to induce ER stress, resulting in hepatocyte pathogenesis. This study suggested that ER stress-mediated transcription factor XBP1 is specifically associated to upregulate HBV transcription upon G1806A core promoter variation.

## Discussion

In this study, the effect of mutations in the core promoter on HBV DNA gene expression was investigated. The development of HCC is caused by various factors such as route of viral transmission, the immune response of the host and the interaction of numerous transcription factors [18, 28]. Previous studies showed that HBV transcription was regulated by transcription factors such as C/EBP, FOXA, HNF1, HNF4 and AP-1 [13, 19, 29]. C/EBPβ is part of the C/EBP family, which is also a known nuclear factor for IL-6 (NF-

IL-6) for IL-6 production [1]. Hepatic C/EBPβ induction is known to regulate inflammation in the liver cell though production of various cytokines including IL-1β, IL-6, IL-8, IL-12 and TNFα [22]. Therefore, C/EBPβ binding to mutation sites in the HBV core promoter results in the induction of the inflammatory response in the liver, leading to development of chronic hepatitis.

Chronic hepatitis B therapies lead to reduce liver damage and HCC production by reducing viral copy number and inhibiting HBV DNA replication [11]. A variety of nucleotide analogues inhibits reverse transcription by HBV DNA polymerase and plays a critical role in the inhibition of viral replication and the normalization of ALT levels. Long-term maintenance therapy of nucleotide analogues is essential for continued antiviral effects against HBV, however nucleotide analogues treatment often induce drug-resistance mutation of HBV gene.

Both A1762T and G1764A mutations are the most common mutations in the core promoter, associating with HCC development. The double mutation expression reduced precore RNA transcription and HBeAg expression. In addition, it moderately increased genome replication through up regulation of pgRNA levels [8, 21, 25]. In addition to A1762T/G1764A, mutations can be detected at nearby positions such as 1753, 1757, 1766, and 1768. Site-directed mutagenesis experiments have suggested that the additional mutations at 1753, 1766, and 1788 further reduce HBeAg expression and enhance genome replication, with the A1762T/G1764A/C1766T triple mutant having greater than 10 folds higher replication capacity than the wild-type virus [26]. In this study, we newly reported that two core promoter mutation, C1713T and G1806A, were closely related to upregulate the core promoter activity by producing novel promoter-specific transcription factor, C/EBPβ and XBP1, respectively.

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## The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

## References

1. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. and Kishimoto, T. 1990. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. *EMBO J.* **9**, 1897-1906.
2. Allweiss, L. and Dandri, M. 2017. The role of cccDNA in HBV maintenance. *Viruses* **9**, 156.
3. An, P., Xu, J., Yu, Y. and Winkler, C. A. 2018. Host and viral genetic variation in HBV-related hepatocellular carcinoma. *Front. Genet.* **9**, 261.
4. Beck, J. and Nassal, M. 2007. Hepatitis B virus replication. *World J. Gastroenterol.* **13**, 48-64.
5. Belyhun, Y., Liebert, U. G. and Maier, M. 2018. Analysis of HBV basal core promoter/precore gene variability in patients with HBV drug resistance and HIV co-infection in Northwest Ethiopia. *PLoS One* **13**, e0191970.
6. Block, T. M., Guo, H. and Guo, J. T. 2007. Molecular virology of hepatitis B virus for clinicians. *Clin. Liver Dis.* **11**, 685-706.
7. Brunetto, M. R., Oliveri, F., Coco, B., Leandro, G., Colombatto, P., Gorin, J. M. and Bonino, F. 2002. Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study. *J. Hepatol.* **36**, 263-270.
8. Buckwold, V. E., Xu, Z., Chen, M., Yen, T. S. and Ou, J. H. 1996. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J. Virol.* **70**, 5845-5851.
9. Chang, M. H. 2007. Hepatitis B virus infection. *Semin. Fetal Neonatal Med.* **12**, 160-167.
10. Chong, C. L., Chen, M. L., Wu, Y. C., Tsai, K. N., Huang, C. C., Hu, C. P., Jeng, K. S., Chou, Y. C. and Chang, C. 2011. Dynamics of HBV cccDNA expression and transcription in different cell growth phase. *J. Biomed. Sci.* **18**, 96.
11. Conjeevaram, H. S. and Lok, A. S. 2003. Management of chronic heaptitis B. *J. Hepatol.* **38,** 90-103.
12. Costa, R. H., Kalinichenko, V. V., Holterman, A. X. and Wang, X. 2003. Transcription factors in liver development, differentiation, and regeneration. *Hepatology* **38**, 1331-1347.
13. Custer, B. 2004. Global epidemiology of hepatitis B virus. *J. ClinGastroenterol.* **38**, S158-168.
14. Datta S. 2008. An overview of molecular epidemiology of hepatitis B virus (HBV) in india. *Virol. J.* **5**, 156.
15. Hsu, Y. S., Chien, R. N., Yeh, C. T., Sheen, I. S., Chiou, H. Y., Chu, C. M. and Liaw, Y. F. 2002. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology* **35**, 1522-1527.
16. Hunt, C. M., McGill, J. M., Allen, M. I. and Condreay, L. D. 2000. Clinical relevance of hepatitis B viral mutations. *Hepatology* **31**, 1037-1044.
17. Kamijo, N., Matsumoto, A., Umemura, T., Shibata, S., Ichikawa, Y., Kimura, T., Komatsu, M. and Tanaka, E. 2015. Mutations of pre-core and basal core promoter before and after hepatitis B e antigen seroconversion. *World J. Gastroenterol.* **21**, 541-548.
18. Kao, J. H., Wu, N. H., Chen, P. J., Lai, M. Y. and Chen, D. S. 2000. Hepatitis genotypes and the response to interferon therapy. *J. Hepatol.* **33**, 998-1002.
19. López-Cabrera, M., Letovsky, J., Hu, K. Q. and Siddiqui,

A. 1991. Transcriptional factor C/EBP binds to and transactivates the enhancer element II of the hepatitis B virus. *Virology* **183**, 825-829.
20. McMahon, B. J. 2009. The natural history of chronic hepatitis B virus infection. *Hepatology* **49**, S45-55.
21. Moriyama, K., Okamoto, H., Tsuda, F. and Mayumi, M. 1996. Reduced precore transcription and enhanced core-pregenome transcription of hepatitis B virus DNA after replacement of the precore-core promoter with sequences associated with e antigen-seronegative persistent infections. *Virology* **226**, 269-280.
22. Poli, V. 1998. The role of C/EBP isoforms in the control of inflammatory and native immunity functions. *J. Biol. Chem.* **273**, 29279-29282.
23. Pollicino, T., Bellinghieri, L., Restuccia, A., Raffa, G., Musolino, C., Alibrandi, A., Teti, D. and Raimondo, G. 2013. Hepatitis B virus (HBV) induces the expression of interleukin-8 that in turn reduces HBV sensitivity to interferon-alpha. *Virology* **444**, 317-328.
24. Quarleri, J. 2014. Core promoter: a critical region where the hepatitis B virus makes decisions. *World J. Gastroenterol*. **20,** 425-435.
25. Scaglioni, P. P., Melegari, M. and Wands, J. R. 1997. Biologic properties of hepatitis B viral genomes with mutations in the precore promoter and precore open reading frame. *Virology* **233**, 374-381.
26. Tsai, A., Kawai, S., Kwei, K. Gewaily, D., Hutter, A., Tong, D. R., Li, J., Wands, J. R. and Tong, S. 2009. Chimeric constructs between two hepatitis B virus genomes confirm transcriptional impact of core promoter mutations and reveal multiple effects of core gene mutations. *Virology* **387**, 364-372.
27. Wang, J., Huang, H., Liu, Y., Chen, R., Yan, Y., Shi, S., Xi, J., Zou, J., Yu, G., Feng, X. and Lu, F. 2020. HBV genome and life cycle. *Adv. Exp. Med. Biol.* **1179**, 17-37.
28. Wu, J. F. and Chang, M. H. 2015. Natural history of chronic hepatitis B virus infection from infancy to adult life-the mechanism of inflammation triggering and long-term impacts. *J. Biomed. Sci*. **22**, 92.
29. Zheng, Y., Li, J. and Ou, J. H. 2004. Regulation of hepatitis B virus core promoter by transcription factors HNF1 and HNF4 and the viral X protein. *J. Virol.* **78,** 6908-6914.

## 초록 : B형 간염 바이러스의 ntC1731T 및 G1806A의 core 프로모터 돌연변이에 의한 HBV 유전자 발현 증가 분석

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B형간염바이러스(HBV)의 만성 감염은 간경화와 간세포암 발생 빈도를 현저히 높인다. HBV 감염의 임상적 결과는 숙주 유전적 요인과 바이러스의 유전자 변이, 그리고 환경적 요인 등에 결정된다. HBV 복제를 위한 HBV의 pre-genomic RNA 전사는 바이러스의 core promoter 활성화에 의해 조절된다. Core promoter 돌연변이는 급성 간 질환과 간세포암 발생에 연관되어 있다. 본 연구팀은 미얀마의 HBV 감염 환자들로부터 바이러스 유전자를 획득하여 core promoter 부위의 유전자 변이들을 파악하였다. Core promoter의 상대적 유전자 활성 차이를 분석하기 위해서 core promoter를 luciferase reporter에 재조합한 시스템을 제작하였다. 분석한 core promoter의 유전자 변이들 중에서 C1731T와 G1806A 돌연변이가 HBV core promoter의 전사 활성화를 증가에 관여하였다. 돌연변이 부위를 중심으로 전사 인자들의 가능한 결합 부위 변화를 컴퓨터 프로그램 분석을 통해 조사한 결과, C/EBPβ와 XBP1 반응 부위가 새롭게 생성되었음을 도출하였다. C/EBPβ의 세포 내 발현은 C1713T 돌연변이를 가진 core promoter의 전사 활성을 증가시켰으며, XBP1 발현은 G1806A 돌연변이를 함유한 M95 promoter를 활성을 증가시켰다. HBV 감염의 치료는 약제 내성과 백신 회피 돌연변이 발생으로 문제점을 가지고 있는 상황에서, 이 연구 결과는 HBV core promoter 돌연변이의 분자생물학적 그리고 임상학적 중요성을 제공한다.