

B Precore/Core DNA

.

epitope

B (Hepatitis B Virus, HBV) .

가 HBV 3.2 Kb DNA

, HBV 4 open reading frames(ORFs) 가 .

가 ORFs S(surface)

1). HBV ,

T P(polymerase) , X

Chisari2) 가 HBV , nucleocapsid core

3-5) . precore core 6).

HBV Precore core HBe HBc

, ,

, HBV HBV

HBV가 HBV replication complex packaging .

HBe HBc

. .

HBV 가

, 가

가 . 7,8). HBV

가 T (,

가 ,)

, HBV precore core

, precore core

HBV HBV ,

B

: 1998 8 17

: 1998 12 9

'97

97-B-1-0003)

(98-4) .

(#HMP- .

1. Specimen DNA

HBV

HBV -20

2. DNA

DNA Kaneko 9)

90 μ l microtube , 1 N

NaOH 10 μ l 0.1 N 37

60 12,000 rpm 30

1 N HCl 10 μ l NaOH

10 boiling 1

, 12,000 rpm 5

polymerase chain reaction (PCR)

3. PCR HBV DNA

PCR primer Fujiyama 10)

HBV subtype adr4 (Accession No. X01587)

() (Bioneer,

) . Precore/core

sense antisense primer BamHI

XbaI cloning .

DNA primer

Table 2 . PCR

. PCR tube 10X reaction

buffer (Promega) 5 μ l, 10 mM dNTP 4 μ l, 5 U/ μ l Taq

polymerase 0.25 μ l, 50 μ M primer 1 μ l,

33.75 μ l, template 5 μ l PCR

Perkin Elmer 480 PCR .

PCR 94 3 1 cycle , 94

60 , 56 60 , 72 60 30 cycles

, 72 5 extension .

PCR 10 μ l 2 μ l gel loading

buffer(Type I) ethidium bromide가

1 % agarose gel loading 100 V 20

. gel UV transi-

lluminator DNA band

, DNA band pcDNA3 plasmid cloning

4. PCR HBVc DNA cloning

PCR HBVc DNA 14 μ l

BamHI XbaI (Boehringer Mannheim) 2 μ l, 10X

buffer (Buffer A) 2 μ l 37 4

. PCR 1 % low

melting agarose 15 UV

transilluminator 754 bp DNA band

microtube 80 5 gel

ligation . pcDNA3 plasmid

(Invitrogen) ligation

. Ligation 10X ligation buffer

1 μ l, pcDNA3 plasmid 4 μ l, HBV

DNA 3 μ l, ligase 2 μ l 16 overnight

. Ligation Top10F' transformation

ampicillin LB plate (50ug/ml)

transformants selection , colony PCR

HBVc DNA가 plasmid

5.

Sequenase version 2.0 sequenc-

ing kit(USB) . Wizard miniprep DNA

purification kit (Promega) plasmid DNA

5 ug DNA 0.1 volume 2 N NaOH

37 30 incubation denatura-

tion , 0.1 volume 3 M sodium acetate (pH

5.2) 가 , 2 volume ethanol 가

- 70 15 12000 rpm, 4 , 5 DNA

70% ethanol DNA pellet ,

. DNA pellet 7 μ l 5X

reaction buffer 2 μ l, sequencing primer (10 μ M) 1 μ l

가 annealing, labeling (35S-dATP), ter-

mination reation stop solution

boiling water 2 ice

denaturation . 6%

urea (7.5 M) polyacrylamide gel (1800 V,

70 W, 1st run 90) . gel 3MM

Whatman paper wrap , 80

60 gel , . gel X-ray

Table 1. Clinical and serological datas in 7 patients with chronic HBV infection

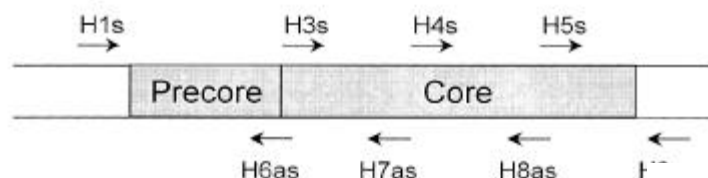
Pt. No.	Age/sex	Histological diagnosis*	HBVeAg/antibody	HBV DNA	Abnormal ALT
1	45/M	LC	- / +	+	-
2	38/F	CAH+LC	- / +	+	-
3	42/M	LC	+ / -	+	-
4	40/M	CAH+LC	+ / -	++	+
5	48/F	CAH	- / +	+	+
6	21/M	CAH	+ / -	++	+
7	44/M	CAH	+ / -	+	+

* LC, liver cirrhosis ; CAH, chronic active hepatitis.

Table 2. Primers used for amplification and sequencing of HBV precore and core DNA

Name	Primer Sequences
H1s	GTAAGGATCCGTTTACCAGCACCATGCAA
H2as	GAGCTCTAGAGTTTCCCACCTTATGAGTCC
H3s	ATGGACATTGACCCGTATAA
H4s	CCTCACCATACAGCACTCAG
H5s	TTGTGGTTTTCATATTTCTGT
H6as	AGAGGCGGTGTCGAGGAGAT
H7as	CTAATTCCCTGGATGCTGG
H8as	GAGGCGAGGGAGTTCTTCTT
T7s	TAATACGACTCACTATAGGG
SP6as	ATTTAGGTGACACTATAG

Primer positions on HBVc DNA



, 24 autoradiography
DNA . DNA
Ausbel 11

1. HBV

(chronic active hepatitis, CAH)
3 , (liver
cirrhosis, LC) 2 , 2 ,

7 precore core
HBV(HBVc) DNA

cloning

5 , 2 , 21
48 41.2 . HBe
4 , anti-HBe 3 3
7+ seroconversion 12
alanine transaminase (ALT)
7 4 7+ ,
3

(Table 1). HBVc DNA
sense antisense primer

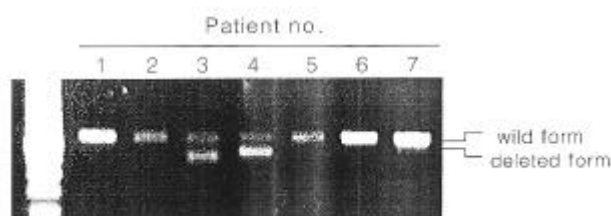
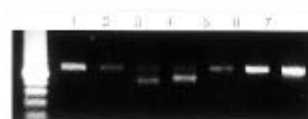


Fig 1. Wild type and deletion mutants of HBV DNA encoding precore and core region. Serum of seven chronic active hepatitis patient were used for amplification of HBV DNA by PCR.

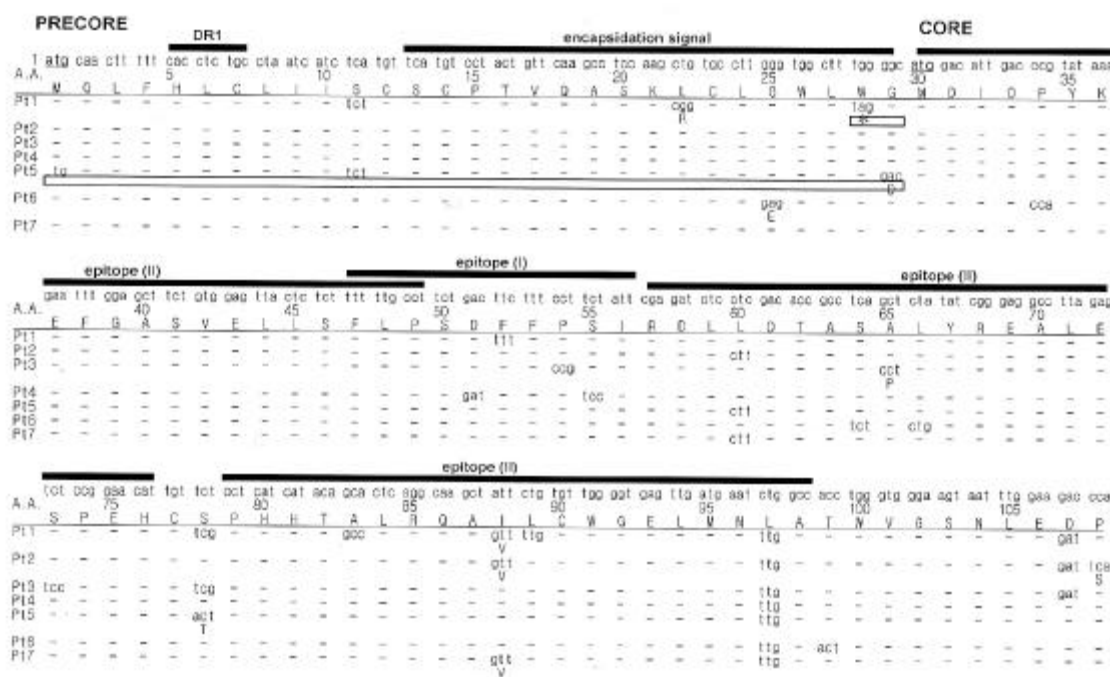


Fig. 2 Nucleotide and amino acid sequences of precore and core gene of HBV isolated from chronic active hepatitis patients with or without liver cirrhosis. Open boxes represent deleted sequences, and [] represents stop codons.

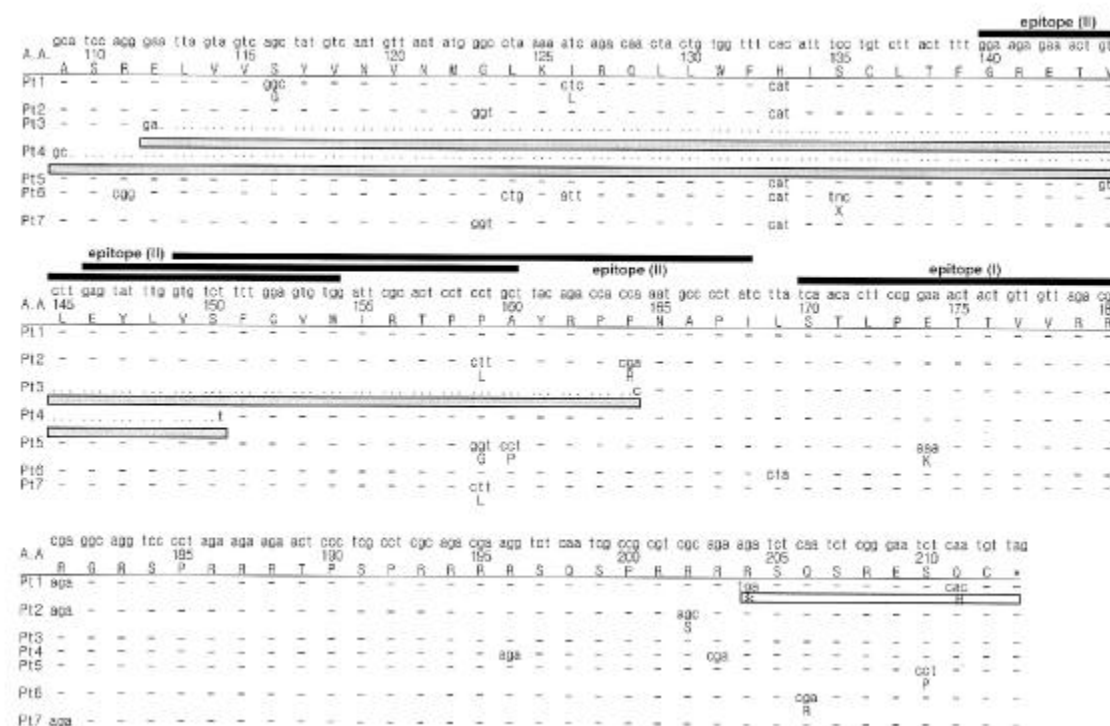


Fig. 2. (continued) Nucleotide and amino acid sequences of precore and core gene of HBV isolated from chronic active hepatitis patients with or without liver cirrhosis. Open boxes represent deleted sequences, and [represents stop codons.

- 4 : B Precore/Core DNA -

(Table 2), B nonsense mutation, 10 silent mutation

NaOH DNA PCR , 2 11 5

HBVc DNA 1% agarose gel missense mutation 6 silent mutation, 3

. 7 5 7 1 missense mutation

HBVc DNA band 3 6 silent mutation . 4 5

4 가 (deletion) silent mutation, 5 12

156 bp 123 bp가 DNA band가 5 missense mutation, 6

(Fig. 1). 13 3 missense mutation

10 silent mutation, 7 7

2. Precore core 2 missense mutation

(Fig. 2).

HBVc DNA

DNAsis Prosis program(Hitachi Co.)

Fujiyama HBV core

(HBV subtype adr4, Accession No. X01587)

DNA

missense mutation

silent mutation ,

가 (deletion) . 3

336 - 491 , 4 327 - 449 가

. Fig. 2

.

7 B precore

1

5 2 , 6

. 1 83 guanine

adenine 28 codon

tryptophan (TGG) codon (TAG)

27 precore

, 5 codon

ATG adenine 가 1

29 , precore 가

. 1

5 HBe anti-HBe

, HBV DNA

(Table 1). Precore DR1 encapsidation

signal 6 HBV DNA 가

1 5 HBV DNA가

.

Precore core 1

16 4 missense mutation 2

Precore HBe Carman 8)

HBe

precore 가 ,

precore 가 6). Precore

HBe 12, 13), core 14)

, pregenomic RNA encapsidation signal15, 16)

HBV life cycle

. precore 가

B

6). 1

5 , HBV precore

HBe

, HBV

precore HBV

Core HBV core 가

pregenomic RNA packaging HBc

6).

3 4 156 bp 123

bp가 DNA band가

HBV core DNA 가

. 3 336 -

491 (A.A. 112 - 164) , 4 327

- 449 (A.A. 109 - 150) ,

가 A.A. 112 - 150

Wakita 17)

. Wakita 17)

HBV core DNA (immune tolerance)
 , HBe , 2) ,
 HBV core 가 6) 가 15 35
 , HBe 가 HBV 가
 가 21) HBV
 . HBV core 가
 Ferrari 18) HBV core
 A.A. 79 - 98 A.A. 146 - 160 , 10%
 (Fig. 2)가 가 immunodominant T epitope 가
 , 3 4 HBV . HBV HBV
 core A.A. 146 - 160 DNA polymerase proofreading
 가 , 가 fidelity가 error 가 (10-5/base/year)
 HBV core 가
 HBV core 가
 HBV 가 가
 HBV HBV
 . HBV , mutant polypeptide
 DNA core
 missense mutation 2) . Roingeard 22) Gunther 23)
 . HBe HBV (gene product)
 ALT 가 , HBV
 6 7 HBV core (abberant gene product, HBe
 13 7 , missense mutation HBc) 가
 3 2 ,
 Bozkaya 19) , ALT T , CD8
 HBe HBV (+) CD4 (+) T 가 T
 HBV 가 가 (immune 가
 clearance phase) HBV core .
 . HBV core MHC HBV peptide가
 HBV core T 가
 . HBV core
 viral antigen/peptide presentation
 Chuang 20) . 1, 2, 7 88
 HBV , isoleucine valine
 HBV 가 , Akarca 21) valine
 가 isoleucine HLA-1 10
 . T (CTL) 가
 가 HBV 5 210
 , serine proline viral
 , HBV packaging/assembly HBV
 가 HBV T

mutant clone , B
HBV pool HBV core 가
T clonal selection HBV core 가 HBV
(immunological selective pressure) HBV core DNA
(1)
, (2)
cytokine

= Abstract =

**Mutations in the Pre- C and Core Region
of Hepatitis B Virus DNA in Chronic
HBV Carriers**

Su Yul Ahn, M.D.

*Department of Internal Medicine,
Kosin University School of Medicine, Pusan, Korea*

1Jong Wook Park, M.D., Su Jeong Yun, M.D.
2Byung Kil Choe, M.D. and 3Min Ho Suh, M.D.

*1Department of Immunology, 2Institute for Medical
Science, 3Department of Microbiology,
Keimyung University School of Medicine, Pusan, Korea*

Objective : Causes of diverse clinical sources of patients with chronic hepatitis B virus(HBV) infection are not fully-known. The host immune response to HBV antigen and the appearance of mutant viruses are believed to be important factors. To determine whether appearance of precore and core mutant viruses are related to the clinical course of the patients, we analysed the entire core region of viral DNA in 7 HBV chronic carriers.

Methods : Serum was obtained from 7 patients(3 chronic active hepatitis, 4 CAH with cirrhosis)and pre-C and core region of HBV were amplified by polymerase chain reaction, then directly sequenced.

Result : In all 7 HBV DNA there was a point mutation from T to C at nucleotide 2104 of core region, and each DNA also contained 6 to 17 variable point mutations at different nucleotides yielding various amino acid substitution. One of DNA had a point mutation from A to G at nucleotide 1898, converting tryptophan(TGG) to a stop codon(TAG). Two cases of deletion mutations covered C-region segment ranging from nucleotide 2142 to 2306 and one case of deletion covered pre-C region ranging from nucleotide 1815 to 1843.

Conclusion : Three out of seven DNA contained

: HBV precore core
가
,
가 HBV core
B
가
: 7 B
NaOH PCR HBV
core DNA . HBV core DNA pcDNA3
plasmid cloning recombinant plasmid
sequencing . DNA sequencing
Sanger dideoxynucleotide chain termination
DNAsis program (Hitachi
Co.) . HBV core
Prosis program(Hitachi Co.)
: 7 5 HBV core DNA
band 2
157bp 122bp가 DNA band , 1
precore
open reading frame
nonsense mutation
, missense
mutation .
: B HBV

mutational sites coincided with known immunodominant T cell epitopes and rest of the mutational sites could also affect the antigenicity of the HBV. Therefore, mutant HBV could alter the host immune response, and may modulate the clinical course of infection.

Key Words : Chronic Hepatitis B, HBV pre core/core DNA mutation

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