Association between single nucleotide polymorphisms in deoxycytidine kinase and lamivudine monotherapy response among patients with chronic hepatitis B

Hyun Woong Lee

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Kwang-Hyub Han

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of Hyun Woong Lee is approved.

Thesis Supervisor: Kwang-Hyub Han

Min Goo Lee: Thesis Committee Member#1

Jin Sung Lee: Thesis Committee Member#2

Byong Ro Kim: Thesis Committee Member#3

Sang Hoon Ahn: Thesis Committee Member#4

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Author

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<ABSTRACT>

Association between single nucleotide polymorphisms in deoxycytidine kinase and lamivudine monotherapy response among patients with chronic hepatitis B

Hyun Woong Lee

Department of Medicine

The Graduate School, Yonsei University

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Background and Aims: Deoxycytidine kinase (dCK) is the important rate-limiting enzyme of the intracellular phosphorylation of lamivudine to its active triphosphates. This study was tried to confirm the known dCK polymorphisms in Chinese and Caucasian, to discover new dCK polymorphisms in Korean and to evaluate whether the discovered single nucleotide polymorphisms (SNPs) of dCK gene is associated with the treatment outcomes of lamivudine monotherapy in patients with chronic hepatitis B (CHB).

Methods: All dCK genes were sequenced from 24 healthy volunteers and 24

patients with CHB for discovering the SNPs. A total of 125 CHB patients were enrolled to compare the treatment outcomes of lamivudine according to genetic variants in dCK gene. They all treated with lamivudine 100mg once daily for at least 48 weeks. At week 48, those who achieved virological and biochemical response with undetectable HBV DNA and ALT normalization were classified as good response. Those who were occurred primary non-response or virologic breakthrough at 48 weeks were considered as poor response.

Results: In Korean, 15 SNPs of dCK gene were detected. Among them, 7 SNPs, had been previous reported in public SNP database. Eight novel dCK SNPs were found (g.-2052C>A, g.-360C>G, c.364C>T (P122S), IVS3-46G>*del*., IVS4+40G>T, IVS5+39T>C, IVS5-72A>T, and c.966~975T_{10}>T_{11}). 2 SNPs within the promoter region (g.-360C>G and g.-201C>T) and 3 SNPs (c.364C>T, IVS6+41T>A, and c.948T>C) were in strong linkage disequilibrium, respectively (r^2>0.9). Especially, the allele frequencies of two SNPs (g.-360C>G and g.-201C>T) were more frequent than what has been reported from Caucasians and Chinese (26% vs. 2% and 15.6%). At week 48, those with g.-360CG/g.-201CT and g.-360GG/g.-201TT compound genotype displayed more favourable virological response to lamivudine monotherapy than those with g.-360CC/g.-201CC (70.6% vs. 48.2%, P = 0.027). The allele frequency of g.-360G/g.-201T was significantly higher in good response group (20.0% vs. 9.3%, P = 0.021). In multivariate

analysis, the genotype containing g.-360G/g.-201T haplotype was independent factor for virological response [odds ratio (OR), 2.846; 95% confidence interval (CI), 1.121 – 7.227, *P* = 0.028). In addition, specific haplotype cluster 5-SNP-B (GTCTT), which was g.-360 C>G, g.-201C>T, c.-364C>T (P122S), IVS6+41T>A, and c.948T>C (3'UTR), was highly associated with good response in patients with CHB, while haplotype cluster 5-SNP-C (CCTAC) was associated with poor response (OR, 2.830; 95% CI, 1.035 – 7.737, *P* = 0.043).

Conclusions: These results anticipate that SNP haplotypes of dCK gene may play an important role as a genetic marker for predicting lamivudine responsiveness in patients with CHB.

Key words : deoxycytidine kinase, single nucleotide polymorphisms, chronic hepatitis B, lamivudine

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I. INTRODUCTION

The recent development of new and potent anti-viral agents may offer many therapeutic options against chronic hepatitis B virus (HBV) infection. Coincidently, antiviral resistance has become an increasingly common problem during long-term treatment with anti-viral agent. Recently, multi-drug resistant HBV has been reported in patients who received sequential treatment with nucleoside/tide analog (NA) monotherapy.[1-3] Therefore, an understanding of the mechanisms of drug resistance is important for preventing the emergence of resistance and designing new drugs.[4]

NAs replace natural nucleosides during the synthesis of the first or second strand (or both) of HBV DNA. They function as competitive inhibitors of the viral reverse transcriptase and DNA polymerase.[5] Because NAs partially and reversibly suppress viral replication, they must be given for more than 1 year in most cases to achieve maximal efficacy. Unfortunately, a long duration of NA treatment is associated with an increasing risk of emergence of drug resistance.[6]

Up to recently, lamivudine has been considered as first-line therapy for individuals with non-cirrhotic and cirrhotic liver disease. Nevertheless, the efficacy of lamivudine is limited by the development of drug-resistant HBV mutants, restricting its utility as a long-term therapy for chronic hepatitis B (CHB).[7] Numerous studies have shown that resistance to antiviral agent such as lamivudine is the major cause of treatment failure. Although viral factors and poor compliance are the most important factors in treatment failure, another important factor to be considered is cellular factor affecting anti-viral efficacy. However, it remains unclear if any of these mechanisms play a role in the response to anti-HBV drugs.[8,9]

The cellular factors of drug resistance include host genetic factors and ability to efficiently convert the agent to its active metabolite. Over the past decade, the relationship between the genetic composition and drug responses has become an important focus of medical practice, especially with the discovery of single nucleotide polymorphisms (SNPs) in genes encoding

receptors and target enzymes mediating drug effects or enzymes directly implicated in drug metabolism.[10-14]

NAs are prodrugs that must be metabolized intracellularly to exert their activity. The nucleoside-based reverse transcriptase inhibitors (NRTIs) approved for anti-viral agents include compounds that mimic endogenous pyrimidine or purine nucleosides. They need to be activated for their anti-viral activity via a phosphorylation process to their nucleoside triphosphates or nucleoside diphosphate that functions as the inhibitor of polymerase.[15-18] One or more steps in sequential phosphorylation of NRTIs from initial monophosphate to diphosphate to triphosphate formation can be rate limiting in formation of active drug. Usually, the initial phosphorylation step is the rate-limiting step in the activation process and may elucidate some of differences in potency among the various NAs.[19]

To date, three different kinases are known to be involved in mediating DNA synthetic process leading to lamivudine phosphorylation. Among them, deoxycytidine kinase (dCK) is considered as the important enzyme because phosphorylation into its monophosphate form is initiating step for the activation of lamivudine.[20,21] The dCK gene is located on chromosome 4q13.3, spanning over 37 kb and containing seven exons. With an open reading frame of 783 bp, its protein product functions as a homodimer of 60 kDa, consisting of two subunits of 30.5 kDa.[22-24] Recently, inactivated dCK transcripts due to alternative splicing were reported in acute myeloid leukemia (AML) patients

resistant to chemotherapy.[25] Another report suggested that specific SNPs in dCK gene affecting variations in gene expression would be considered as a new mechanism contributing to the resistance to 1-β-arabinofuranosylcytosine (Ara-C) in AML patients.[26] However, there was no analysis demonstrated that the dCK gene contributes to the development of lamivudine resistance.

Therefore, the potential influence of a genetic variation such as SNPs of the dCK gene on clinical sensitivity to lamivudine is worthy of investigation. The objectives of this study are to confirm the known dCK polymorphisms in Chinese and Caucasian, to discover new dCK polymorphisms in Korean and to evaluate whether the discovered SNPs of dCK gene is associated with the treatment outcomes of lamivudine therapy in patients with CHB.

II. PATIENTS AND METHODS

1. Study population

A total of 125 patients with CHB, who visited Severance hospital between Jan. 2002 and Dec. 2003 and were treated with lamivudine 100mg for at least 48 weeks, were enrolled in the current study. Patients enrolled in the study meet the following entry criteria: they were 18–75 years of age; the presence of serum HBsAg was observed for at least 6 months; they had elevated serum alanine aminotransferase (ALT) on two occasions, at least 1 month apart, with an average value of ≥ 2 times the upper limit of normal (ULN); the presence of serum hepatitis B e antigen (HBeAg) and HBV DNA had been documented on two occasions, at least 1 month apart. Additional requirements included: a hemoglobin value of ≥ 10 g/dl, a platelet count of ≥ 70,000 mm^3, a white cell count of ≥ 3,000 mm^3, a polymorphonuclear count of ≥ 1,500 mm^3 and normal renal function with normal serum creatinine levels. Candidates were required to have compensated liver disease with a prothrombin time of less than 4 sec, prolonged over control values, a serum albumin of ≥ 3.0 g/dl, a total bilirubin of ≤ 4 mg/dl, and no history of hepatic encephalopathy or bleeding esophageal varices and imaging features suggestive of cirrhosis on ultrasonography.

The exclusion criteria were as follows: a history of corticosteroid treatment

within 6 months of entry; previous therapy with IFN; the presence of antibody to human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis D virus (HDV); a history of malignancy, evidence of other forms of liver disease; or a history of intravenous drug abuse. Patients with other significant medical or psychiatric problems were also excluded.

Serum HBeAg, anti-HBe, HBV DNA and ALT were tested every 3 or 6 months, and whenever necessary during medication and after drug cessation. In addition, peripheral blood samples from 112 healthy donors were also used as control. Patients and controls were compared for genotype and allele frequencies of the SNPs of the dCK gene.

2. Definitions

Patients without decline in serum HBV DNA by $\geq$ 1 $\log_{10}$ copies/mL after the first 6 months of therapy were classified as "primary non-response (NR)." Virologic breakthrough (VB) was defined as patients with increase in serum HBV DNA by > 1 $\log_{10}$ copies/mL (10-fold) above nadir after achieving virological response, during continued treatment.[27] Those who achieved virological and biochemical response with undetectable HBV DNA and ALT normalization at least 48 weeks were classified as good response. By contrast, those who were classified as NR and were occurred VB at 48 weeks were considered as poor response.

3. Assay methodology

Commercially available enzyme-linked immunoadsorbent assay (ELISA) was used for the detection of serum HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe (Abbott Laboratories, North Chicage, IL, USA). The HBV DNA was detected by Digene Hybrid Capture II Assay (Digene Diagnostics Beltsville, MD, USA; detection limit, 0.5 pg/ml = 1.4 x 10^5 copies/mL).

4. Sequence analysis of dCK gene

24 healthy volunteers and 24 patients with CHB were screened for discovering the SNPs of the dCK gene. A 5-ml aliquot of EDTA blood was collected from each individual. Genomic DNA from peripheral blood stored samples will be isolated using a NucleoGen Genomic DNA isolation Kit (NucleoGen, Seoul, Korea) according to the manufacturer's instructions and subsequently stored at 4℃ until analysis. 13 primer sets were used to amplify all seven dCK exons and the core promoter region (Table 1). Several base pair fragments of the dCK gene were amplified with forward primers and reverse primers in a final volume of 25㎕ containing 50~100 ng of genomic DNA, 20 pmol of each primer, 0.2 mM dNTPs, 2 U of *Taq* polymerase (iNtRON biotechnology, Seoul, Korea) and manufacturer's standard polymerase chain reaction (PCR) buffer. Amplification was performed in a GeneAmp PCR

system 9700 (Perkin Elmer Corp., Norwalk, CT, USA). PCR conditions were 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 sec, 55℃ for 30 sec and 72℃ for 50 sec, with final extension at 72℃ for 7 min. PCR products were identified by electrophoresis, then PCR products were purified with PCR purification kit (iNtRON biotechnology, Seoul, Korea) and analyzed by direct sequencing (bionex Co. Ltd, Seoul, Korea).

Table 1. PCR primers and amplification conditions used for dCK

Region	Strand	PCR-product forward primer	Reverse primer
Promoter1	Forward	5' - CAG GAA ACA GCT ATG ACC CAG CCA GAA ACT GGT AGA AC - 3'	5' - TGT AAA ACG ACG GCC AGT TGA CTC CAT CCT CAG AAA TAA - 3'
Promoter2	Forward	5' - CAG GAA ACA GCT ATG ACC TGA TTC TTG CTG TTT AAT CCT - 3'	5' - TGT AAA ACG ACG GCC AGT GAC CTA ATG AGG CAA GAG AA - 3'
Promoter3	Forward	5' - CAG GAA ACA GCT ATG ACC CTG CCT AGT CTT GCC TAG AA - 3'	5' - TGT AAA ACG ACG GCC AGT CCA GAA ATA CCG TCA TTA GC - 3'
Exon1	Reverse	5' - CAG GAA ACA GCT ATG ACC CTA GAG AGG CGG GTT TTC - 3'	5' - TGT AAA ACG ACG GCC AGT GTA AGG GAA GGA TGC TCT G - 3'

Exon2	Forward	5' - CAG GAA ACA GCT ATG ACC GGT GGC CAT TAG GAG TAT TA - 3'	5' - TGT AAA ACG ACG GCC AGT GAA ACC CAT TGA TAT GGA GA - 3'
Exon3	Forward	5' - CAG GAA ACA GCT ATG ACC ATC ATC TTG GTT TTG CTG AT - 3'	5' - TGT AAA ACG ACG GCC AGT CAC CAT ATT CCC AAC AGT TT - 3'
Exon4	Reverse	5' - CAG GAA ACA GCT ATG ACC AAG TAA TCT GGC CTC TCA CA - 3'	5' - TGT AAA ACG ACG GCC AGT TAA TTT AGA GGT GGG GAG TG - 3'
Exon5	Forward	5' - CAG GAA ACA GCT ATG ACC TGT GGA TGG ATA CCA AAA AT - 3'	5' - TGT AAA ACG ACG GCC AGT TCA TTG CAA TCA AGA GAA TG - 3'
Exon6	Forward	5' - CAG GAA ACA GCT ATG ACC CCC AGC TGT ATA TAT TTT GTA CC - 3'	5' - TGT AAA ACG ACG GCC AGT CCG AAG GAT CTT TAT TTT AGC - 3'
Exon7-A	Forward and Reverse	5' - CAG GAA ACA GCT ATG ACC AGC TGG GGT CTT CAA CTA TT - 3'	5' - TGT AAA ACG ACG GCC AGT TAA GAA ACT GGT CAC CAA CG - 3'

Exon7-B	Forward	5' - CAG GAA ACA GCT ATG ACC TTT CTC ATA GCA GGA AAT GTA G - 3'	5' - TGT AAA ACG ACG GCC AGT TTA ATG GAT GCT TTC TAG CC - 3'
Exon7-C	Forward	5' - CAG GAA ACA GCT ATG ACC TAT CCT GAA AGC ATT ATT TTT - 3'	5' - TGT AAA ACG ACG GCC AGT TGA TTA TTA CAT CTT TTT AGA ACT G - 3'
Exon7-D	Forward	5' - CAG GAA ACA GCT ATG ACC TTT TTA GTT TGT TTT TGT TTG G - 3'	5' - TGT AAA ACG ACG GCC AGT ACC TTT CTA GGA GAG CAA ACT - 3'

PCR program with an annealing temperature of 55℃

5. Statistical analysis

All data were expressed as means ± standard deviation (SD). Student's t test compared mean age, pretreatment ALT levels and HBV DNA levels. The chi-square test or Fisher's exact test compared the sex ratio, and frequencies of novel and known dCK genotype. Based on the gene frequencies, predicted phenotype frequencies were calculated according to the Hardy-Weinberg equation and compared with the observed frequencies using the chi-square test. We assessed all variables using the logistic regression model. Cumulative virologic breakthrough rates and HBeAg loss rates were estimated using Kaplan-Meier method and compared by log-rank test. All statistical analysis was performed using Statistical Package for Social Science (SPSS Inc. Chicago, Illinois.) version 12.0. A *P* value of less than 0.05 was considered significant.

III. RESULTS

1. Identification of SNPs in dCK gene in the Korean population

All the 7 coding exon of dCK and 1.5kb of the proximal promoter were analyzed in a group of 24 healthy volunteers and 24 patients with CHB. A total of 15 variants were identified in 48 Korean. Among them, four SNPs were present in the 5' regulatory region (the first nucleotide upstream of the translation initiation site being defined as -1 position), which were g.-2052C>A, g.-1329C>T, g.-360C>G, and g.-201C>T with allele frequencies of 0.979:0.021, 0.979:0.021, 0.74:0.26, and 0.734:0.266, respectively. Three SNPs were detected in exons which were c.364C>T (P122S), c.948T>C, and c.966-975T_{10}>T_{11} with allele frequencies of 0.969:0.031, 0.979:0.021, and 0.979:0.021, respectively. In addition, we detected 7 SNPs in introns, and 1 SNP in 3' UTR, respectively. To detect population structure, an additional 14 SNPs were chosen from the National Center for Biotechnology Information (NCBI) public database. However, they were not detected in Korean. Among 15 SNPs, which were detected in 48 Korean, 7 SNPs had been previous reported in public SNP database (NCBI reference number). Interestingly, eight novel dCK SNPs were found (g.-2052C>A, g.-360C>G, c.364C>T (P122S), IVS3-46G>*del.*, IVS4+40G>T, IVS5+39T>C, IVS5-72A>T, and c.966~975T_{10}>T_{11}) (Table 2). No significant deviation from Hardy-Weinberg expectations occurred in 24

healthy volunteers and 24 patients with CHB in 15 SNPs. Especially, the two promoter SNPs g.-360C>G and g.-201C>T were previously confirmed in Caucasians and Chinese. They were in perfect linkage disequilibrium and found at an allele frequency of 26%. The allele frequencies of two SNPs were more frequent than what has been reported from Caucasians and Chinese (26% vs. 2% and 15.6%). Linkage analysis revealed significant disequilibrium between g.-360C>G and g.-201C>T (r-squre 1.0, D' 1.0). One SNP is non-synonymous and results in the substitution of proline at amino acid position 122 by serine (c.364C>T in exon 3, P122S).

6 SNPs were selected to compare for genotype and allele frequencies between lamivudine response groups. Three promoter SNPs and one intronic SNP, which were g.-1329C>T, g.-360C>G, g.-201C>T and IVS6+41T>A were selected according to high frequencies of alleles. Two SNPs located in exon 3 and 3'UTR were selected (c.364C>T, and c.948T>C) (Fig. 1)

Table 2. Positions, sequences and frequencies of dCK variations (N=48*)

Position	SNP name †	Nucleotide sequence	Allele frequency ‡	OH	EH	HWE *p*
Promoter	g.-2052C>A §	5' – TCC TCT CGG TAG CTG CCA GTA CT[C/A] TTA CCA TTT CAT GAG TTT TCC CC - 3'	C:A=0.979:0.021	0	0.041	0.0211
Promoter	g.-1431A>G	5' – AAC CAA GTG CTT CAA GAG TCC CA[A/G] TTG CAG TGT TCT TAA GGG TTA CT - 3'	A:G=1:0	0	0	1
Promoter	g.-1359A>G	5' – TAG GTG CCC TTT ACA GTC GTC CA[A/G] TTG GTT GCA CCC TAT GAA GGA TT - 3'	A:G=1:0	0	0	1
Promoter	g.-1329C>T	5' – GCA CCC TAT GAA GGA TTG GCC TG[C/T] GACC AAT CAG AGG CTG AAA TGG A - 3'	C:T=0.979:0.021	0.042	0.041	1
Promoter	g.-360C>G §	5' – CCT CTT CCC GCG CCC TGC CCG GG[C/G] GCC TGG CTG CTT GGG GTA GAG GC - 3'	C:G=0.74:0.26	0.271	0.385	0.0824
Promoter	g.-201C>T	5' – CGG CTT GAG GAG GGC GGG GCC GC[C/T] CCG CAG GCC CGC CAG TGT CCT CA - 3'	C:T=0.734:0.266	0.277	0.39	0.092
Intron 1	IVS1+37G>C	5' – GGA AAT GTG GGA CGC AAG GCT GG[G/C] GTG TCG CGG CAG TGG CTG AAG CT - 3'	G:C=1:0	0	0	1
Intron 1	IVS1-174G>A	5' – ACA AAT GTG CTT AAT GAA ATT GG[G/A] CAG	G:A=0.969:0.031	0.062	0.061	1

		GGA GCC TTT TCA TTT TCT TC - 3'				
Intron 1	IVS1-110T>G	5' – AAA GTT TAG AAA GTT GAA TGT TT[T/G] GGG CTT TTT ATG TTG CTT GCT AT - 3'	T:G=1:0	0	0	1
Intron 2	IVS2+114G>A	5' – CTT TCA AAT CTC GCT TTA GGT AT[G/A] TAT CTT CAT CTA GGT GGT GGT TA - 3'	G:A=0.969:0.031	0.062	0.061	1
Exon 3	A100A	5' – TCT TTT ACC TTC CAA ACA TAT GC[C/T] TGT CTC AGT CGA ATA AGA GCT CA - 3'	C:T=1:0	0	0	1
Exon 3	P122S[§]	5' – GCA AGC TCA AAG ATG CAG AGA AA[C/T] CTG TAT TAT TTT TTG AAC GAT CT - 3'	C:T=0.969:0.031	0.062	0.061	1
Intron 3	IVS3-46G>*del.*[§]	5' – GTT CTC TTT TTT ATT TCT TTG TT[G/-] TTT TTT TTT GAA ATG ATA CAT GT - 3'	G:*del*.=0.978:0.022	0.043	0.043	1
Intron 3	IVS3-45T>*del.*	5' – TTC TCT TTT TTA TTT CTT TGT TG[T/-] TTT TTT TTG AAA TGA TAC ATG TG - 3'	T:*del*.=1:0	0	0	1
Intron 4	IVS4+40G>T[§]	5' – ATG TGT TTC ACT GAA AAT TTA AA[G/T] AAA TAT TTA GAA CTC TTT TCA GT - 3'	G:T=0.989:0.011	0.022	0.022	1
Intron 5	IVS5+39T>C[§]	5' – ATT TAC TAT TCA TTT TAA ATA CC[T/C] TTG TTA CCT TTG TTA AAT TTT AA - 3'	T:C=0.99:0.01	0.021	0.021	1
Intron 5	IVS5-72A>T[§]	5' – TTT GAT TTT CCA AGG ACA TAC GA[A/T] TGA ATT TTT AAT GTT TCT CTC TT - 3'	A:T=0.99:0.01	0.021	0.021	1

Intron 7	IVS6+41T>A	5' – ACA AAC AAA TAT TTG TTT TTT CT[A/T] AAA AAG TGT ACT GAG TGG TGA GA - 3'	T:A=0.969:0.031	0.062	0.061	1
Exon 7 (3' UTR)	c.948T>C	5' – GAA TCT TAT GCA AAA CTT TTT GA[T/C] CAG TTT CTT TTC TTT TGT TTT TT - 3'	T:C=0.979:0.021	0.042	0.041	1
Exon 7 (3' UTR)	c.966~975T_{10}>T_{11} §	5' – TTT GAY CAG TTT CTT TTC TTT TG[T_{10}/T_{11}] AAA AAA GAC ATT TAA AGA CAA AG - 3'	T_{10}/T_{11}=0.979:0.021	0.042	0.041	1
Exon 7 (3' UTR)	c.1796A>G	5' – TTT AGA AAA TTT TAT GTA TTT TA[A/G] AAT AAG GGG AAG AGT CAT TTT CA - 3'	A:G=1:0	0	0	1
Exon 7 (3' UTR)	c.1797A>G	5' – TTA GAA AAT TTT ATG TAT TTT AA[A/G] ATA AGG GGA AGA GTC ATT TTC AC - 3'	A:G=1:0	0	0	1
Exon 7 (3' UTR)	c.1827*del*.>A	5' – GAA GAG TCA TTT TCA CTT TTA AA[-/A] CTA CTA TTT TTC TTT CCA AGT CA - 3'	*del*.:A=1:0	0	0	1
Exon 7 (3' UTR)	c.1904*del*.>C	5' – TAA TTT AGT GGA TTA ACC AGT CC[-/C] AGA CGC ACT GAT CTT TGC AAA GG - 3'	*del*.:C=1:0	0	0	1
Exon 7 (3' UTR)	c.1952A>C	5' – GAC TTA ATT TCA AAT CTG TAA TT[A/C]CCA TAC ATA AAC TGT CTC ATT AT - 3'	A:C=1:0	0	0	1
Exon 7 (3' UTR)	c.2031A>G	5' – GTA TAA ATT AAT TTG TTA ATT AA[A/G] TAT TTC TTA AGT ATA AAC CTT AT - 3'	A:G=1:0	0	0	1
Exon 7	c.2105A>T	5' – AAT GTA ATT TCA GTT CTA AAA AG[A/T] TGT AAT	A:T=1:0	0	0	1

(3' UTR)		AAT CAT TTT AGA ATT AA - 3'				
Exon 7 (3' UTR)	c.2254TT>*del.*	5' – AAC CTT TAA AAA TGT ATA ATG AC[TT/-] TTA AAA TTT GTA GAA TTG AAA AG - 3'	TT:*del*..=1:0	0	0	1
3' UTR	c.2419A>T	5' – TCA TGT CTT CCA TTA GAA TTT AT[A/T] TAT TGC TCT TTA TAG TTT GCT CT - 3'	A:T=0.978:0.022	0.043	0.043	1

*24 healthy volunteers and 24 patients with CHB were screened for the SNP of the dCK gene

†Base pair position numbered from translation initiation site

‡Frequency for the variant genotype

§ Eight novel dCK SNPs were found (g.-2052C>A, g.-360C>G, P121S, IVS3-46G>*del.*, IVS4+40G>T, IVS5+39T>C, IVS5-72A>T, and 966~975T_{10}>T_{11})

Abbreviation: SNP, Single nucleotide polymorphism; EH, Expected heterozygous; OH, Observed heterozygous; HWE p, Hardy-Weinberg equilibrium p-value

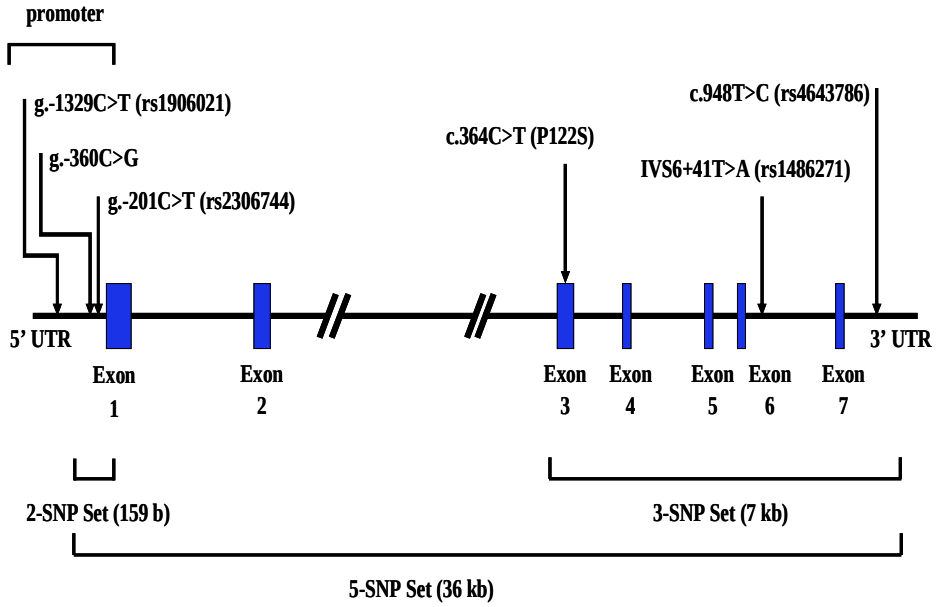


Fig. 1. Human deoxycytidine kinase (dCK) gene structure and single nucleotide polymorphism (SNP) sites for the haplotype SNP sets studied.

The size of the gene is over 37 kilobases(kb). 6 SNPs were selected to compare for genotype and allele frequencies between lamivudine response groups. Three promoter SNPs and one intronic SNP, which were g.-1329C>T, g.-360C>G, g.-201C>T and IVS6+41T>A were selected according to high frequencies of alleles. Two SNPs located in exon 3 and 3'UTR were selected (c.364C>T, and c.948T>C).

2. Correlation between dCK SNP genotypes and the treatment responses with lamivudine in 125 chronic hepatitis B patients

The 125 CHB patients were divided into two groups according to their responses to lamivudine monotherapy. 69 patients achieved good responses which are virological response with undetectable HBV DNA and ALT normalization at least 48 weeks. On the contrary, 56 patients showed poor responses, including 9 patients who did not have decline in serum HBV DNA by ≥ 1 $\log_{10}$ copies/mL after the first 6 months of therapy, 47 patients who had increase in serum HBV DNA by > 1 $\log_{10}$ copies/mL (10-fold) above nadir after achieving virological response at 48 weeks.

The univariate analysis of clinical and virological factors showed that younger age ($P = 0.030$) and higher serum ALT levels ($P = 0.002$) were associated with a higher probability of good response. There were no obvious differences in clinical data such as sex, baseline serum HBV DNA, and so on, before treatment between the two groups (Table 3).

The relationship between six dCK SNP genotypes and the treatment responses was analyzed (Table 4). Interestingly, there was statistically significant difference between the two groups in terms of the distribution of genotype and allele frequency of the g.-360C>G, g.-201C>T. Allele frequency analysis of the g.-201C/T and g.-360C/G in 125 CHB patients, as well as 112 peripheral blood samples from normal individuals, is in agreement with the

Hardy-Weinberg equilibrium (HWp = 0.699). Sequence analysis showed that g.-360C/g.-201C and g.-360G/g.-201T were in perfect linkage disequilibrium (Fig. 2.)

Multivariate logistic regression analysis was performed using one categorized value according to dCK SNP genotype and three continuous values that were previous known as predictive factors of good response. Age, baseline serum ALT level, HBV DNA level and the genotypes containing g.-360G/g.-201T haplotype were independent predictive factors for good response (Table 5).

Table 3. Clinical characteristics of 125 patients with chronic hepatitis B

Valuables	Good Response N = 69 (%)	Poor Response N = 56 (%)	*P* Value
Male: Female	51 : 18	44 : 12	0.544
Mean age (years)	41.5 ± 10.3	45.8 ± 11.3	0.030
Baseline serum ALT (IU/L)	188.5 ± 107.7	136.3 ± 78.0	0.002
Baseline HBV DNA (log_{10} copies/mL)	7.7 ± 1.1	8.1 ± 1.0	0.074
Initial HBeAg positive	57 (82.6)	52 (92.9)	0.088
Previous IFN-α therapy	10 (15.9)	2 (4.4)	0.071
Mean treatment duration (months)	31.8 ± 19.4	32.4 ± 19.4	0.864
Mean follow-up duration (months)	47.7 ± 20.4	45.4 ± 18.8	0.528

NOTE. Values are given as mean ± standard error of the mean

Abbreviation: ALT, alanine aminotransferase; HBV, hepatitis B virus; IFN-α, interferon alpha

Table 4. Genotype distribution and allele frequency of g.-1329C>T, g.-360C>G, g.-201C>T, c.364C>T, IVS6+41T>A, and c.948T>C in two groups of chronic hepatitis B[*]

Valuables		Good Response[†] N = 69 (%)	Poor response[†] N = 56 (%)	*P* Value
g.-1329C>T		55 (79.7)	45 (80.4)	
Genotype distribution	CC	53 (96.4)	43 (95.6)	1.000
	CT + TT	2 + 0 (3.6)	2 + 0 (4.4)	
Allele frequency	C	108 (98.2)	88 (97.8)	1.000
	T	2 (1.8)	2 (2.2)	
g.-360C>G		65 (94.2)	54 (96.4)	
Genotype distribution	CC	41 (63.1)	44 (81.5)	0.027
	CG + GG	22 + 2 (36.9)	10 + 0 (18.5)	
Allele frequency	C	104 (80 0)	98 (90.7)	0.021
	G	26 (20.0)	10 (9.3)	
g.-201C>T		65 (94.2)	54 (96.4)	
Genotype distribution	CC	41 (63.1)	44 (81.5)	0.027
	CT + TT	22 + 2 (36.9)	10 + 0 (18.5)	

Allele frequency	C	104 (80 0)	98 (90.7)	0.021
	T	26 (20.0)	10 (9.3)	
c.364C>T (P122S)		64 (92.8)	54 (96.4)	
Genotype distribution	CC	58 (90.6)	44 (81.5)	0.148
	CT + TT	6 + 0 (9.4)	8 + 2 (18.5)	
Allele frequency	C	122 (95.3)	96 (88.9)	0.064
	T	6 (4.7)	12 (11.1)	
IVS6+41T>A		64 (92.8)	54 (96.4)	
Genotype distribution	TT	58 (90.6)	44 (81.5)	0.148
	TA + AA	6 + 0 (9.4)	8 + 2 (18.5)	
Allele frequency	T	122 (95.3)	96 (88.9)	0.064
	A	6 (4.7)	12 (11.1)	
c.948T>C		55 (79.7)	44 (78.6)	
Genotype distribution	TT	50 (90.9)	37 (84.1)	0.302
	TC + CC	5 + 0 (9.1)	6 + 1 (15.9)	
Allele frequency	T	105 (95.5)	80 (90.9)	0.199
	C	5 (4.5)	8 (9.1)	

[*] 125 patients with CHB who were treated with lamivudine were retrospectively analyzed.

[†]Good response: virological response with undetectable HBV DNA and ALT normalization at 48 weeks. Poor response: virological response without decline in serum HBV DNA by $\geq$ 1 $\log_{10}$ copies/mL after the first 6 months of therapy and with increase in serum HBV DNA by > 1 $\log_{10}$ copies/mL (10-fold) above nadir after achieving virological response, during continued treatment.

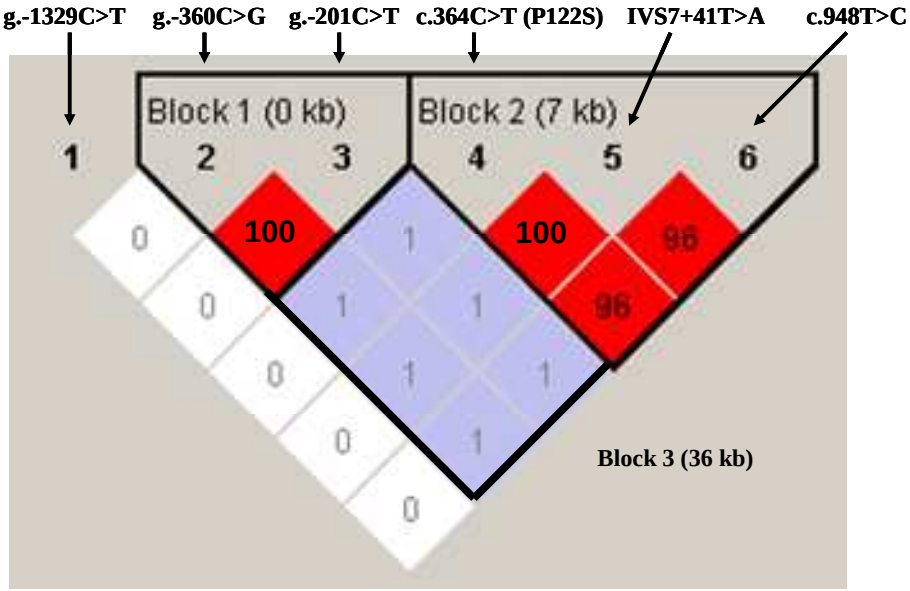


Fig. 2. Linkage disequilibrium (LD) plot of dCK in samples with chronic hepatitis B patients and healthy control

LD plots were generated in Haploview using genotype data from 125 patients with chronic hepatitis B and 112 healthy control samples. The scheme is: black when $r^2 > 0.9$; shades of grey light $0 < r^2 < 0.9$. SNPs that are linked $r^2 > 0.9$ are categorized in the same haplotype block. We used 2-SNP (g.-360C>G and g.-201C>T), 3-SNP (c.364C>T, IVS6+41T>A, and c.948T>C), and 5-SNP (g.-360C>G, g.-201C>T, c.364C>T, IVS6+41T>A, and c.948T>C) sets to create the windows for performing each of 3 separate analyses

Table 5. Multivariate logistic regression analysis of predictive factors associated with good response

Valuables	Odds ratio	95% CI	*P* Value
Genotypes containing g.-360G/g.-201T haplotype	2.846	1.121 – 7.227	0.028
Age (years)	0.962	0.925 – 1.000	0.047
Baseline serum ALT (IU/L)	1.007	1.002 – 1.013	0.005
Baseline HBV DNA (log_{10} copies/mL)	0.564	0.371 – 0.857	0.007

Abbreviation: CI, confidence interval; ALT, alanine aminotransferase; HBV, hepatitis B virus

3. Cumulative virologic breakthrough rates between the groups according to dCK SNP genotypes

Because 9 patients who were classified as NR were excluded, the genotype of two SNPs (g.-360C>G and g.-201C>T) was available in only 116 CHB patients. With a median follow-up of 48 (12 – 90) months, the 3-year cumulative virologic breakthrough rates for the group of g.-360CG/g.-201CT (N = 32) and g.-360GG/g.-201TT (N = 2) were 49.9%, whereas that for the g.-360CC/g.-201CC (N = 82) was 65.8%. Cumulative virologic breakthrough rates of the two groups were presented in Fig. 3 using Kaplan-Meier method. Although those of the genotypes containing g.-360G/g.-201T haplotype were slightly lower, there was no statistically significant difference between two groups ($P = 0.127$).

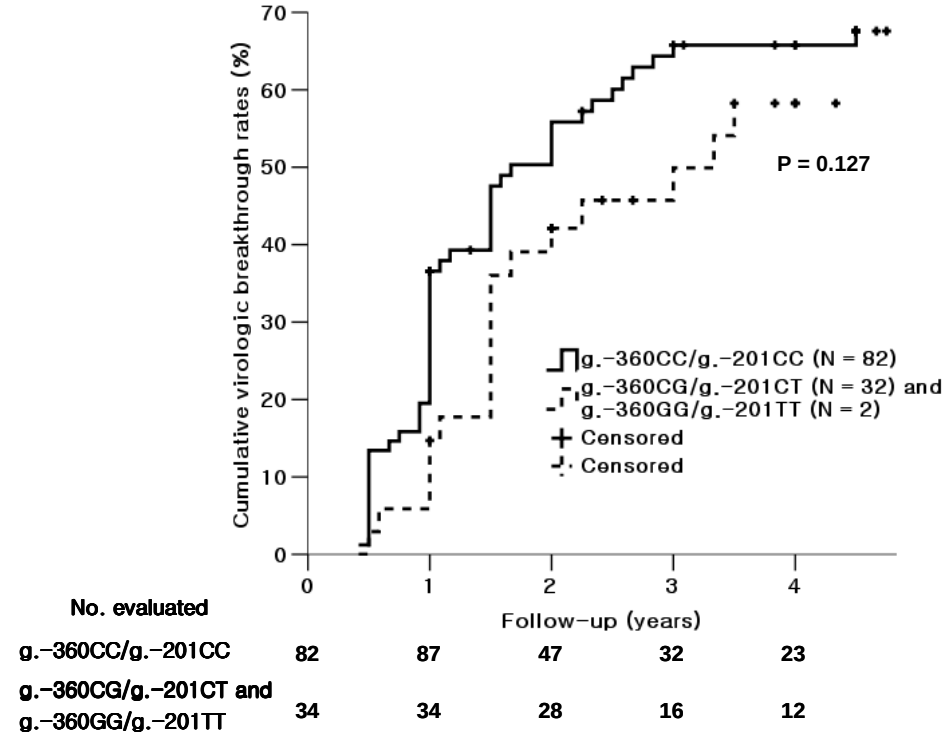


Fig. 3. Cumulative virologic breakthrough rates between the group of g.-360G/g.-201T haplotype and g.-360C/g.-201C haplotype by using Kaplan-Meier method.

The genotype of two SNPs was available in only 116 CHB patients. With a median follow-up of 48 (12 – 90) months, the 3-year cumulative virologic breakthrough rates for the group of g.-360CG/g.-201CT (N = 32) and g.-360GG/g.-201TT (N = 2) were 49.9%, whereas that for the g.-360CC/g.-201CC (N = 82) was 65.8%. Although those of the genotypes containing g.-360G/g.-201T haplotype were slightly lower, there was no statistically significant difference between two groups (P = 0.127).

4 Cumulative HBeAg loss rates between the groups according to dCK SNP haplotypes

Among 125 patients, 19 patients with HBeAg negative CHB were excluded. Allele frequency analysis of the g.-201C/T and g.-360C/G in 106 HBeAg positive CHB patients, as well as 112 peripheral blood samples from normal individuals, is also in agreement with the Hardy-Weinberg equilibrium (HWp = 0.533). The 106 HBeAg positive CHB patients were divided into two groups according to loss of HBeAg during lamivudine monotherapy. 41 patients achieved HBeAg loss during treatment period, whereas another 65 patients did not achieve HBeAg loss. Unfortunately, there was no significant difference between the two groups in terms of the distribution of SNP genotype and allele frequency (P = 0.187, P = 0.154) (Table 6).

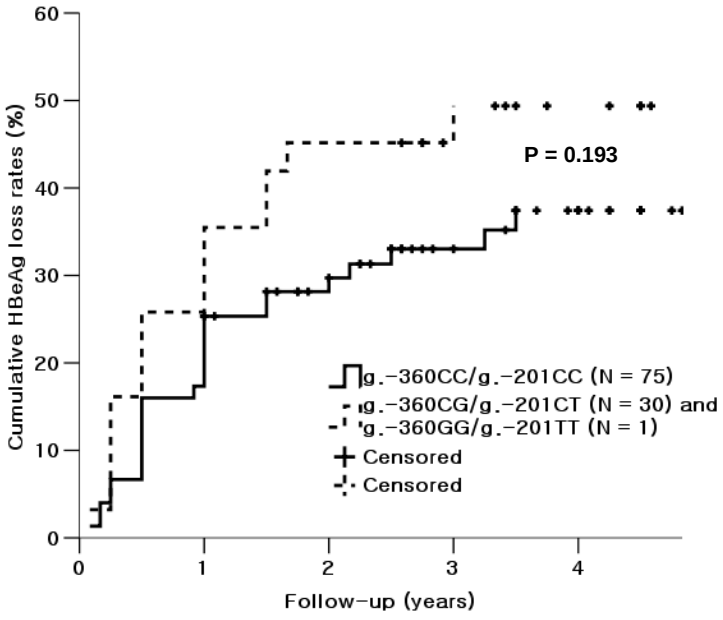
The follow-up data of cumulative HBeAg loss rates were available for 106 HBeAg positive CHB patients. With a median follow-up of 51 (12 – 90) months, the 3-year cumulative HBeAg loss rate for the group of g.-360CG/g.-201CT (N = 30) and g.-360GG/g.-201TT (N = 1) was 49.4%, whereas that for the g.-360CC/g.-201CC (N = 75) was 33.0%. Cumulative HBeAg loss rates of the two groups were presented in Fig. 4 using Kaplan-Meier method. Although those of the genotypes containing g.-360G/g.-201T haplotype were slightly higher, there was no statistically significant difference between two groups (P = 0.193).

Table 6. Genotype distribution and allele frequency of g.-360C/G and g.-201C/T in two groups according to loss of HBeAg during lamivudine treatment[*]

Drug response[†]	Genotype distribution (%)			Allele frequency (%)		
HBeAg loss	CC	CG + GG	*P* Value	C	G	*P* Value
Yes (N = 41)	26 (63.4)	14 + 1 (36.6)	0.187	66 (80.5)	16 (19.5)	0.154
No (N = 65)	49 (75.4)	16 + 0 (24.6)		114 (87.7)	16 (12.3)	

[*] 106 patients with HBeAg positive CHB who were treated with lamivudine were analyzed.

[†]HBeAg loss: achieving loss of HBeAg during continued treatment.



No. evaluated					
g.-360CC/g.-201CC	75	75	54	44	31
g.-360CG/g.-201CT and g.-360GG/g.-201TT	31	31	20	17	12

Fig. 4. Cumulative HBeAg loss rates between the group of g.-360G/g.-201T haplotype and g.-360C/g.-201C haplotype by using Kaplan-Meier method.

The follow-up data of cumulative HBeAg loss rates were available for 106 HBeAg positive CHB patients. With a median follow-up of 51 (12 – 90) months, the 3-year cumulative HBeAg loss rate for the group of g.-360CG/g.-201CT (N = 30) and g.-360GG/g.-201TT (N = 1) was 49.4%, whereas that for the g.-360CC/g.-201CC (N = 75) was 33.0%. Although those of the genotypes containing g.-360G/g.-201T haplotype were slightly higher, there was no statistically significant difference between two groups (P = 0.193).

5. Haplotype structure between control and patients with chronic hepatitis B

We used three SNP sets for haplotype-based association analyses, grouping SNPs on the basis of the level of LD strength. We used 2-SNP (g.-360C>G and g.-201C>T), 3-SNP (c.364C>T, IVS6+41T>A, and c.948T>C), and 5-SNP (g.-360C>G, g.-201C>T, c.364C>T, IVS6+41T>A, and c.948T>C) sets to create the windows for performing each of 3 separate analyses (Fig. 1, 2). In 2-SNP haplotype block, there were 2 configuration (A: CC; and B: GT) that accounted for 100% of all chromosomes in patients with CHB. The haplotype pattern did not differ significantly between control and patients. In addition, specific haplotypes in the 3-SNP and 5-SNP block did not differ significantly between two groups (Table 7).

Table 7. Haplotype clusters and frequencies of 3 single nucleotide polymorphism sets at the dCK gene in patients with chronic hepatitis B[*] and control samples[†]

Haplotype Cluster	SNP[‡]					Frequency		Chi Square	*P* value
	g.-360C>G	g.-201C>T	c.364C>T	IVS6+41T>A	c.948T>C	Control	Patients		
2-SNP Cluster									
A	C	C				0.862	0.835	0.594	0.441
B	G	T				0.138	0.165	0.594	0.441
Total						1.0	1.0		
3-SNP Cluster									
A			C	T	T	0.929	0.924	0.036	0.850

B			T	A	C	0.071	0.071	0.0	0.990
Total						1.0	0.995		
5-SNP Cluster									
A	C	C	C	T	T	0.790	0.759	0.612	0.434
B	G	T	C	T	T	0.138	0.165	0.598	0.439
C	C	C	T	A	C	0.071	0.071	0.0	0.990
Total						0.999	0.995		

*125 patients with CHB who were treated with lamivudine were analyzed.

†Peripheral blood samples from 112 healthy donors were also used as control

‡Two promoter, two exon and one intronic SNPs, which were g.-360C>G, g.-201C>T, c.364C>T, c.948T>C and IVS6+41T>A were selected according to high frequencies of alleles.

6. Haplotype structure and association with lamivudine response among patients with chronic hepatitis B

Using all 5 available SNPs, we simultaneously evaluated. The specific haplotype in the 5-SNP block differed significantly between good response and poor response. One of the clusters, 5-SNP-B (GTCTT) corresponding to 2-SNP-B (GT), was higher frequency in good response than poor response (0.203 *vs* 0.093; $P = 0.019$; OR, 2.830; CI, 1.035 − 7.737). In contrast, cluster 5-SNP-C (CCTAC) corresponding to 3-SNP-B (TAC), was slightly more abundant in poor response (0.039 *vs* 0.100; $P = 0.063$). In lamivudine monotherapy, these data suggested that haplotype cluster 5-SNP-B (GTCTT) was highly associated with good response in patients with CHB, while haplotype cluster 5-SNP-C (CCTAC) was associated with poor response (Table 8, 9).

Table 8. Haplotype clusters and frequencies of 3 single nucleotide polymorphism sets at the dCK gene in patients with good response and poor response*

Haplotype Cluster	SNP†					Frequency		Chi Square	*P* value	Odds Ratio	95% CI
	g.-360C>G	g.-201C>T	c.364C>T	IVS6+41T>A	c.948T>C	Good Response‡	Poor Response‡				
2-SNP Cluster											
A	C	C				0.797	0.907	5.536	0.019	0.351	0.138 – 0.892
B	G	T				0.203	0.093	5.536	0.019	2.846	1.121 – 7.227
Total						1.0	1.0				

3-SNP Cluster											
A			C	T	T	0.953	0.898	2.649	0.104		
B			T	A	C	0.039	0.100	3.470	0.063		
Total						0.989	0.998				
5-SNP Cluster											
A	C	C	C	T	T	0.750	0.806	1.038	0.308		
B	G	T	C	T	T	0.203	0.093	5.536	0.019	2.830	1.035 – 7.737
C	C	C	T	A	C	0.039	0.100	3.470	0.063		
Total						0.992	0.999				

* 125 patients with CHB who had available DNA sample and were treated with lamivudine were analyzed.

†Two promoter, two exon and one intronic SNPs, which were g.-360C>G, g.-201C>T, c.364C>T, c.948T>C and IVS6+41T>A were selected according to high frequencies of alleles.

‡Good response: virological response with undetectable HBV DNA and ALT normalization at 48 weeks. Poor response: virological response without decline in serum HBV DNA by $\geq$ 1 $\log_{10}$ copies/mL after the first 6 months of therapy and with increase in serum HBV DNA by > 1 $\log_{10}$ copies/mL (10-fold) above nadir after achieving virological response, during continued treatment.

Abbreviation: CI, Confidence interval

Table 9. Multivariate logistic regression analysis of predictive factors associated with good response

Valuables	Odds ratio	95% CI	*P* Value
Specific haplotype cluster (GTCTT)*	2.830	1.035 – 7.737	0.043
Age (years)	0.955	0.916 – 0.997	0.035
Baseline serum ALT (IU/L)	1.007	1.001 – 1.012	0.012
Baseline HBV DNA (log_{10} copies/mL)	0.548	0.344 – 0.873	0.011

*Two promoter, two exon and one intronic SNPs, which were g.-360C>G, g.-201C>T, c.364C>T, c.948T>C and IVS6+41T>A were selected according to high frequencies of alleles.

Abbreviation: CI, confidence interval; ALT, alanine aminotransferase; HBV, hepatitis B virus

IV. DISCUSSION

Novel eight dCK SNPs were found (g.-2052C>A, g.-360C>G, c.364C>T (P122S), IVS3-46G>*del.*, IVS4+40G>T, IVS5+39T>C, IVS5-72A>T, and c.966~975T_{10}>T_{11}). Especially, the two promoter SNPs g.-360C>G and g.-201C>T, which were more confirmed in Caucasians and Chinese, were more frequent in Korean (26% vs. 2% and 15.6%). In addition, we found one SNP (P122S) in exon 3, which leads to change the amino acid sequence. Although Joerger M et al. found these SNPs in Caucasian healthy volunteers, Shi et al. did not find any exon 3 polymorphisms in Chinese study cohort.[26,28]

Deoxycytidine kinase is responsible for the phosphorylation of several deoxyribonucleosides and their analogs. It is the rate limiting enzyme in the activation of many important antiviral drugs.[29] Deficiency of this enzyme activity is associated with resistance to antiviral and anticancer chemotherapeutic agents, whereas increased enzyme activity is associated with increased activation of these compounds to antiviral nucleoside triphosphate derivatives. It is clinically important because of its relationship to drug resistance and sensitivity.[30-32]

According to Shi et al, acute myelogenous leukaemia patients carrying the g.−360CG/g.−201CT or g.−360GG/g.−201TT genotype expressed significantly higher levels of dCK mRNA than patients carrying the g.−360CC/g.−201CC

genotype, and the former also displayed a favourable response to 1-β-arabinofuranosylcytosine (AraC)-containing induction chemotherapy.[26] These results confirm the functionality of the g.−360CG/g.−201CT promoter polymorphism. On the basis of these results, CHB patients carrying the g.−360CG/−201CT or g.−360GG/−201TT genotype may have active lamivudine triphosphate derivatives and show a favorable response to drug sensitivity.

In present study, we explored the potential relationship between the clinical response to lamivudine and the SNPs of the dCK gene, a key enzyme in the metabolism of lamivudine. When CHB patients were divided into two groups according to clinical outcomes (virological and biochemical response at 48 weeks) to lamivudine, there was statistically significant difference between the two groups in terms of the distribution of SNP genotype and allele frequency (P = 0.027, P = 0.021). Also, the genotypes containing g.-360G/g.-201T haplotype were independent predictive factors for good drug response (P = 0.028; OR, 2.846; 95% CI, 1.121 – 7.227). In addition, our data suggested that haplotype cluster 5-SNP-B (GTCTT) was highly associated with good response in patients with CHB (0.203 *vs* 0.093; P = 0.019; OR, 2.830; CI, 1.035 – 7.737). The cumulative HBeAg loss rates showed a tendency to increase for long-term follow-up in CHB patients carrying the g.−360CG/g.−201CT or g.−360GG/g.−201TT genotype. However, there was no statistically significant difference between two groups (P = 0.193).

Shi et al. suggested that g.-360G/g.-201T haplotype may provide the cellular transcriptional machinery with more efficient promoter/enhancer elements.[26] According to this hypothesis, a possible explanation of our results could be that the g.-360C/g.-201C homozygous genotype may be considered as new factor contributing to the resistance to lamivudine.

Recently, Lamba JK et al. reported that the homozygous genotype (c.364CC) of c.364C>T (P122S) had a significantly greater ratio dCK activity to dCK mRNA expression than other subjects (c.364CT or c.364TT). In our data, the frequency of good response was slightly higher in CHB patients with c.364CC genotype than those with c.364CT or c.364TT genotype (Table 4).[33]

Lamivudine is one of effective antiviral agents used in CHB treatment. However, resistance to lamivudine remains a main drawback in the treatment of CHB. Therefore, the main concern with long-term NA therapies is the emergence of drug resistance. Long-term treatment of CHB patients with NA agents may result in failure of therapy, due to the rapid emergence of resistant virus mutants with decreased susceptibility to therapeutic agents. Besides, cellular factors as well as viral resistance may contribute to the weaken efficiency of antiviral agents. This led to the assumption that altered drug metabolism in host cells may contribute to inefficient activation of lamivudine in CHB patients. Thus, intracellular sub-therapeutic levels of the active compounds may develop. In this intracellular environment, selection of resistant virus populations may be promoted.[34,35]

Our study may give an answer to the question, whether cellular factors, in addition to viral mutations, may account for the success or failure of lamivudine monotherapy. It may suggest that enzymatic activation of lamivudine probably may have an effect on antiviral activity in CHB patients.

As other factors affecting antiviral efficacy, the pharmacokinetics properties including, intestinal absorption, distribution into the infected liver, and cellular uptake using nucleoside transporters remain unclear if any of these properties play an important role in the response to antiviral agents. In the clinical setting, host determinants such as compliance, severity of the liver disease and associated disorders may also influence antiviral efficacy.[36,37]

The limitations of this study were due to its small size. Thus the results of the exploratory analysis in CHB patients need to be confirmed in a larger patient population. If the correlation could be confirm in a larger number of patients, the SNPs of dCK gene would be a genetic marker predicting treatment response for antiviral agents. Another perspective is that the data generated in this study must be used to elucidate the functionality of dCK candidate SNPs in patients receiving antiviral agents. Experimental research must be explored to find the exact mechanism of how SNPs regulate dCK gene expression. Lastly, the functionality of exon and intronic SNPs and the relationship between SNPs have to be studied. Although CHB is a very heterogeneous disease with different genotype and viral mutation that are of prognostic significance, the pharmacogenetics of lamivudine could help in better understanding of drug

responsiveness and guide us to develop individualized antiviral therapy in CHB patients receiving NA.

In summary, 15 dCK SNPs were found. Among them, eight dCK SNPs were newly discovered. Especillay, the two promoter SNPs g.-360C/G and g.-201C/T were more frequent in Korean than other ethnic group. We found one SNP (P122S) in exon 3, which leads to change the amino acid sequence. The genotype and the allele frequency of g.-360G/g.-201T were significantly higher in good response. Also, the genotypes containing g.-360G/g.-201T haplotype were independent predictive factors for good drug response. In addition, specific haplotype cluster 5-SNP-B (GTCTT) was highly associated with good response in patients with CHB. These results anticipate that SNP haplotypes of dCK gene may play an important role as a genetic marker for predicting lamivudine responsiveness in patients with CHB.

We have shown in a pilot study of the clinical implication of dCK polymorphisms in CHB patients undergoing treatment with lamivudine. In future, further studies will be tried whether these SNP haplotypes are associated with the levels of transcriptional expression of dCK gene, as well as the level of active NAs triphosphates. Our data will provide important evidence of a relationship between dCK gene and antiviral agents

V. CONCLUSION

We hypothesize that a genetic variation such as SNPs of the dCK gene may influence on the clinical responsiveness to lamivudine monotherapy.

1. Eight dCK SNPs were newly discovered (g.-2052C>A, g.-360C>G, c.364C>T (P122S), IVS3-46G>*del*., IVS4+40G>T, IVS5+39T>C, IVS5-72A>T, and c.966~975T_{10}>T_{11}).
2. Especillay, the two promoter SNPs g.-360C/G and g.-201C/T were more frequent in Korean than other ethnic group.
3. We found one SNP (P122S) in exon 3, which leads to change the amino acid sequence.
4. The genotype and the allele frequency of g.-360G/g.-201T were significantly higher in good response.
5. The genotypes containing g.-360G/g.-201T haplotype were independent predictive factors for good response.
6. The specific haplotype cluster 5-SNP-B (GTCTT) was highly associated with good response in patients with CHB.
7. These results anticipate that SNP haplotypes of dCK gene may play an important role as a genetic marker for predicting lamivudine responsiveness in patients with CHB.

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< ABSTRACT (IN KOREAN)>

만성 B형 간염 환자에서 Deoxycytidine kinase 단일 염기 다형성과 라미부딘 치료반응과의 상관성 연구

<지도교수 한 광 협>

연세대학교 대학원 의학과

이 현 웅

연구배경 및 목적: Deoxycytidine kinase (dCK)는 간세포내에서 라미부딘이 약리작용을 할 수 있는 형태로 인산화하는데 가장 중요한 효소로 알려져 있다. 본 연구의 목적은 서양인과 중국인에서 이미 알려진 dCK 단일 염기 다형성을 확인하며, 한국인에서 새로운 단일 염기 다형성을 발견하고, 발견된 단일 염기 다형성이 만성 B형 간염 환자에서 라미부딘 치료효과와 관련이 있는지를 알아보고자 하였다.

대상 및 방법: 24명의 건강한 사람과 24명의 만성 B형 간염 환자로부터 dCK 유전자를 모두 분석하여 단일 염기 다형성을 평가하였다. 또

한, 라미부딘으로 48주 이상 치료를 받은 125명의 만성 B형 간염 환자를 대상으로 임상자료 분석과 발견된 단일 염기 다형성과의 상관관계를 분석하였다. 라미부딘 치료 48주에 혈청 HBV DNA 가 음전되고 ALT 정상을 획득한 군을 “약물 반응 군”으로, 초기 치료 반응이 없고 치료 48주 이내에 바이러스 돌파가 발생한 군을 “약물 비반응 군”으로 정의하였다.

결과: 한국인의 dCK 유전자에서 확인된 15개의 단일 염기 다형성 중 7개의 단일 염기 다형성은 기존 데이터 베이스에 알려져 있으나 8개의 단일 염기 다형성은 새롭게 발견되었다 (g.-2052C>A, g.-360C>G, c.364C>T (P122S), IVS3-46G>*del.*, IVS4+ 40G>T, IVS5+ 39T>C, IVS5-72A>T, and c.966~975T_{10}>T_{11}). 특히, promoter 위치에서 발견된 g.-360C/G 와 g.-201C/T 단일 염기 다형성은 강한 연관 불균형 관계를 보이고 있으며 대립유전자형(allele)의 빈도는 서양인이나 중국인에 비해 높았다 (26% vs. 2% and 15.6%). 라미부딘 치료 48주에 g.-360CG/g.-201CT 와 g.-360GG/g.-201TT 유전형을 가진 환자 군이 g.-360CC/g.-201CC 유전형을 가진 환자 군에 비해 효과적인 치료 반응을 보였다 (70.6% vs. 48.2%, P = 0.027). 또한, g.-360G/g.-201T 대립유전자형의 빈도가 약물반응 군에서 의미있게 높았다 (20.0% vs. 9.3%, P

=0.021). 다변량 분석에서 g.-360G/g.-201T 일배체형을 포함하는 유전형이 라미부딘 치료 48주 약물 반응의 독립적인 예측인자였다 [odds ratio (OR), 2.846; 95% confidence interval (CI), 1.121 – 7.227; *P* = 0.028)]. 또한, 5개의 단일 염기 다형성으로, g.-360 C>G, g.-201C>T, c.-364C>T (P122S), IVS6+ 41T>A, c.948T>C (3'UTR), 구성된 특이 일배체형 (GTCTT)의 빈도가 약물 반응군에서 의미있게 높았다 (OR, 2.830; 95% CI, 1.035 – 7.737; *P* = 0.043).

결론: 이러한 결과는 만성 B형 간염 환자에서 dCK 유전자의 단일 염기 다형성이 라미부딘 치료반응을 예측하는데 중요한 유전적 표지자 역할을 할 것으로 기대된다.

핵심되는 말 : deoxycytidine kinase, 단일 염기 다형성, 만성 B형 간염, 라미부딘