© Med Sci Monit, 2011; 17(10): PH75-80
PMID: 21959623

www.MedSciMonit.com
Public Health

Received: 2010.10.08
Accepted: 2011.03.01
Published: 2011.10.01

Molecular epidemical characteristics of Lamivudine resistance mutations of HBV in southern China

Si-Yue Li[ABCE], Li Qin[AD], Lei Zhang[B], Xing-Bo Song[B], Yi Zhou[BF], Juan Zhou[CF], Xiao-Jun Lu[B], Ju Cao[E], Lan-Lan Wang[AD], Jun Wang[BC], Bin-Wu Ying[ADE]

Department of Laboratory Medicine, West China Hospital, Sichuan University, Sichuan Province, China

Source of support: The National Natural Science Foundation of China (No. 30900658; No.81071425)

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

PH

Summary

Background: Lamivudine (LMV), as the preferred oral drug for use in treatment of HBV, always results in development of resistance mutations after long-term treatment. In this study we investigated chronic hepatitis B (CHB) patients in southern China to determine whether different HBV genotypes affect the incidence of LMV resistance mutations.

Material/Methods: The study recruited 185 CHB patients living in southern China. Enzyme-linked immunosorbent assay was used to test for HBV serological markers, and HBV DNA was quantified by real-time PCR. Sequencing was performed to detect HBV genotypes and mutations.

Results: There were 49.19% (91/185) CHB patients with HBV resistant to LMV. Only 2 genotypes were found: B and C; 62.16% (115/185) of patients were infected with genotype B HBV and 37.84% (70/185) of patients were infected with genotype C HBV. The incidence rate of LMV resistance was not significantly different between genotype B and C (49.57% *vs.* 48.57%, $P>0.05$). For the mean age and sex ratio, no significant difference was found. The pattern of rtM204I alone was predominantly observed (36.26%, 33/91), followed by rtM204V+rtL180M (23.08%, 21/91). The overall incidence rate of rtM204I mutation in genotype B (45.61%, 26/57) was more frequent than that in genotype C (20.59%, 7/34) (45.61% *vs.* 20.59%, $P<0.05$), but the incidence rate of other mutation patterns was not significantly different between genotypes B and C.

Conclusions: Our results emphasize that a LMV resistance test before treatment is of great importance in rational and optimal CHB therapy.

key words: **Lamivudine resistance • mutations • HBV • HBV genotypes • southern China**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=881965

Word count: 2114
Tables: 4
Figures: 1
References: 30

Author's address: Binwu Ying and Lanlan Wang, Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, P.R China, e-mail: docybw@gmail.com

Background

At present, hepatitis B virus (HBV) infection remains one of the major global public health problems. Over one-third of the world's population (about 2 billion people) has been infected with HBV at some time in their life, and about 350 million of those remain infected [1]. The distribution of HBV throughout the world shows that, unfortunately, China is an area where HBV infection is highly prevalent [2]. According to a 2008 nationwide survey of China by the Ministry of Health (MOH), there were approximately 93 million chronic HBV carriers.

To reduce morbidity and mortality from chronic HBV infection, antiviral treatment is the only effective approach [3]. Current antiviral agents for the treatment of CHB include interferon (IFN) and nucleoside analogues. As a kind of immunomodulator, IFN has been used since the early 1980s in attempts to suppress HBV replication. Although treatment with IFN may lead to a durable response, its unpleasant adverse effects (flu-like symptoms, fatigue, leucopoenia, hair loss, anorexia, etc.), and high cost limited its use [4,5]. Nucleoside analogues such as Lamivudine (LMV), Adefovir, Entecavir and Telbivudine then became the most common drugs used for antiviral therapy. These chemically synthesized drugs can imitate natural nucleosides, and are integrated into newly synthesized HBV DNA, causing chain termination or competitively inhibiting the reverse transcriptase (RT) activity of the viral polymerase to restraint viral replication [6].

LMV is the first safe oral nucleoside analogue approved for the treatment of HBV by the FDA (December 1998) [7], and it has become the clinical drug of first choice because it is effective and well-tolerated, although it requires long-term therapy [4,8,9]. However, long-term treatment may induce mutations [10,11]. Through the sequencing analysis of the HBV gene, a series of mutations associated with LMV at diverse positions in the RT domain have been identified, located in codon positions 180, 204 [12], 173 [13–15], 213and 207 [6], and others. The existence of those mutations may not only cause distinct virological responses in patients, but also cause drug resistance and eventually lead to treatment failure.

The development of different drug resistance mutations was influenced by the characteristics of patients and the HBV itself. However, there have been few investigations on whether different HBV genotypes may affect the incidence of LMV resistance mutations. The aim of the present study was to investigate CHB patients in southern China to determine the prevalence characteristics of HBV genotypes and LMV resistance mutations, the association of HBV genotypes/LMV resistance mutations with the age/sex characteristics of those patients, as well as the relationship between HBV genotypes and LMV resistance mutations.

Material and Methods

Patients

This study was approved by the Ethics Committee of West China Hospital, Sichuan University, and written informed consent was obtained from all participants. Between August 2009 and July 2010, 185 consecutive patients with CHB were recruited from the hepatitis clinic of West China Hospital of Sichuan University. The patients were positive for hepatitis B surface antigen (HBs Ag), had HBV viral loads ⩾3 log copies/ml, and did not have other infectious diseases such as human immunodeficiency virus, hepatitis C or hepatitis D.

Extraction of hepatitis B virus DNA

Blood samples (5–10 ml) were collected from all patients, using disposable syringes under aseptic conditions. Sera were separated and stored in a freezer at –20°C until the time of use. HBV DNA was extracted from 200 μl of serum using Chelex 100 (Ke Hua Bio-Engineering Co. Ltd., Shanghai, China), according to the manufacturer's instructions.

Testing for HBV serological markers and Quantification of HBV DNA

HBsAg was detected by enzyme-linked immunosorbent assay. To measure the quantity of HBV DNA, the Light Cycler® 480 System for Real-Time PCR (Roche Diagnostics, Shanghai, China) was used.

Detection of HBV genotypes and mutations

The amplification conditions of PCR consisted of 42°C for 5 min and 94°C for 5 min, 40 cycles of 94°C for 7 sec, and 60°C for 50 sec, followed by a final primer extension at 72°C for 5 min. Sequencing was performed using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Mutations were detected by using the Hepatitis B Virus Drug Resistance Mutations and Genotypes Detection Kit (Yuan Qi Bio-Medicine Co. Ltd., Shanghai, China) according to the manufacturer's instructions.

The HBV genotypes were determined by using a web-based genotyping tool for viral sequences from the National Center for Biotechnology Information (NCBI). The nucleotide sequences were compared with those of the HBV genotypes at NCBI (nucleotide LOCUS AF286594).

Statistical analysis

Data were analyzed using χ^2 tests for categorical variables and T tests for continuous variables when appropriate. Values were expressed as mean ±SD for normally distributed variables, and median for non-normally distributed variables. The effects of age, sex and HBV genotypes on LMV mutations were estimated as odds ratios (ORs) and 95% confidence intervals (95% CI) using binary logistic regression. All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 17.0 for Windows; SPSS Inc, Chicago, IL, USA). A *P* value of less than 0.05 was considered statistically significant.

Results

Incidence rate of LMV resistance and distribution of mutation patterns

All 185 samples were successfully sequenced. In total, 91 (49.19%) of these LMV resistance substitutions (LMV resistance mutations group), 88 (47.57%) were had no drug resistance mutations (without mutations group), the remaining 6 (3.24%) patients' mutations were irrelevant to LMV.

Table 1. The prevalence of LMV-resistance mutation patterns in southern China.

Mutation patterns	n (N=91)	%
rtM204I	33	36.26
rtM204I+rtL180M	3	3.30
rtM204I+rtV207M	1	1.10
rtM204I+rtV207L	1	1.10
rtM204I+rtV207L/M	1	1.10
rtM204I+rtV173L	1	1.10
rtM204I+rtS213T	4	4.39
rtM204I+rtA181T	1	1.10
rtM204I+rtA181S	1	1.10
rtM204V	1	1.10
rtM204V+rtL180M	21	23.08
rtM204V+rtL180M+rtV173L	3	3.30
rtM204V+rtL180M+rtV173M	1	1.10
rtM204V+rtL180M+rtV207M	1	1.10
rtM204I/V+rtL180M	4	4.39
rtM204I/V+rtL180M+rtS213T	1	1.10
rtL180M+rtA181V	1	1.10
rtV207M	1	1.10
rtS213T	5	5.49
rtA181T	2	2.20
rtA181V	4	4.39

Six LMV-related mutation sites were detected: rt204, rt180, rt207, rt173, rt213, and rt181. The vast majority of patients (78/91, 85.71%) had the mutations at site rt204, and a compensatory mutation at position rt180 was detected in 35 (35/78, 44.87%) of these patients. Rt180 mutation always existed along with abnormal rt204 (35/36, 97.22%), and only 1 was accompanied with rt181. There were 21 mutation patterns (Table 1). It is obvious that the pattern of rtM204I alone was dominantly observed (36.26%), followed by rtM204V + rtL180M (23.08%), while other mutation patterns were rare.

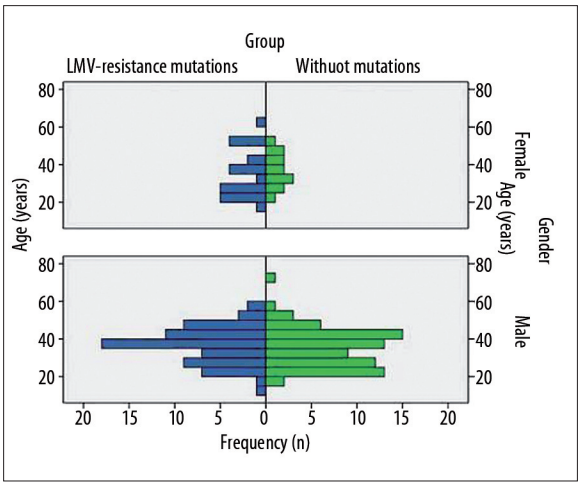

Figure 1. Distribution and frequency of age between two groups according gender.

Age and sex characteristics of patients with LMV-resistance

Age and sex characteristics between LMV-resistant patients and patients without any mutations are shown in Table 2. No significant difference was observed between the 2 groups (age: t=0.434, *P*>0.05; sex: χ^2=3.07, *P*>0.05).

Figure 1 shows the distribution and frequency of age between the 2 groups. There was no significant difference between 2 groups with various age ranges in both females and males.

HBV genotypes isolated from CHB patients in southern China

Only 2 genotypes were identified in 185 patients – B (115, 62.16%) and C (70, 37.84%). For the 91 LMV-resistant individuals, the isolates of genotypes B and C were 57 (62.64%) and 34 (37.36%), respectively. Table 2 shows the incidence rates of LMV resistance were not significantly different between HBV genotypes B and C (χ^2=0.02, P>0.05).

Table 2. Characteristics of CHB patients in different groups.

Variable	Group		Total	*P*
	LMV-resistance mutations	Without mutations		
N	91	88	179	–
Age(means ±SD years)	35.71±10.40	35.05±10.22	–	>0.05
Gender (male/female)	68/23	75/13	–	>0.05
Genotype of HBV (n,%)				
B	57 (50.44)	56 (49.56)	113	>0.05
C	34 (51.52)	32 (48.48)	66	

Table 3. Age and gender characteristics of LMV-resistance patients with different HBV genotypes.

Genotype	n (%)	Age (years) mean ±SD	*P*	Gender(male/female) n	*P*
B	57 (62.64)	35.98±10.77	>0.05	40/17	>0.05
C	34 (37.36)	35.26±9.88		28/6	

Table 4. Relationship between HBV genotypes and LMV-resistance mutations.

Mutation patterns	Genotypes		*P*
	B (n=57)	C (n=34)	
	n (%)	n (%)	
rtM204I	26 (45.61)	7 (20.59)	<0.05
rtM204I+rtL180M	1 (1.75)	2 (5.89)	–
rtM204I+rtV207M	1 (1.75)	–	–
rtM204I+rtV207L	1 (1.75)	–	–
rtM204I+rtV207M/L	–	1 (2.94)	–
rtM204I+rtV173L	1 (1.75)	–	–
rtM204I+rtS213T	3 (5.27)	1 (2.94)	–
rtM204I+rtA181T	–	1 (2.94)	–
rtM204I+rtA181S	–	1 (2.94)	–
rtM204V	1 (1.75)	–	–
rtM204V+rtL180M	14 (24.56)	7 (20.59)	>0.05
rtM204V+rtL180M+rtV173L	–	3 (8.82)	–
rtM204V+rtL180M+rtV173M	–	1 (2.94)	–
rtM204V+rtL180M+rtV207M	1 (1.75)	–	–
rtM204I/V+rtL180M	–	4 (11.76)	–
rtM204I/V+rtL180M+rtS213T	1 (1.75)	–	–
rtL180M+rtA181V	–	1 (2.94)	–
rtV207M	–	1 (2.94)	–
rtS213T	4 (7.04)	1 (2.94)	–
rtA181T	–	2 (5.89)	–
rtA181V	3 (5.27)	1 (2.94)	–

Regression analysis showed that patient age, sex and HBV genotypes did not have significant effects on LMV mutation (OR: 1.01, 95% CI: 0.98–1.04, P=0.64; OR: 0.51, 95% CI: 0.24–1.08, P=0.08; OR: 0.91, 95% CI: 0.49–1.68, P=0.76).

Age and sex characteristics of patients with different HBV genotypes

The age and sex distribution of the 115 patients with genotype B HBV were 35.46±10.73 years and the males/females ratio was 89/26, similar to those of the 70 patients with genotype C, which were (34.94±9.48) years and a male/female ratio of 58/12 (comparing mean age: t=0.332, *P*>0.05; sex: χ^2=0.80, *P*>0.05).

In the 91 patients with LMV resistance mutations, the mean age and sex ratio of patients infected with HBV genotype B were similar to those of patients infected with HBV genotype C. (mean age: t=0.317, *P*>0.05; sex: χ^2=1.67, *P*>0.05) (Table 3).

Relationship between HBV genotypes and LMV resistance mutations

The genotype distributions and frequencies of patients with various mutation patterns were counted, calculated and summarized in Table 4. RtM204I mutation in genotype B (45.61%) was more frequent than that in genotype C (20.59%) (χ^2=5.77, P<0.05); however, the incidence rates

of other mutation patterns were not significantly different between genotypes B and C.

Discussion

LMV, the major oral nucleoside analogue, was approved for the treatment of chronic hepatitis B in China in 1999. However, it has the highest resistance rate in patients receiving long-term therapy [16]. About 20% of patients taking LMV for 1 year develop resistance, and the resistance rate is 70% for patients who take LMV for 5 years [17]. This study showed that the incidence rate of LMV resistance in southern China reached 49.19%, which was much higher than that of other nucleoside analogues (3.24%).

To date, 8 genotypes (A to H) of HBV have been identified world-wide. In China, genotype B and C are predominant. It was previously reported that genotype C is predominant in the regions north of the Yangtze River, while genotype B is more frequent in southern China, and genotypes E, F, G and H were not found in any of the patients studied [18]. In this study we found genotypes B (62.16%) and C (37.84%) in 185 CHB patients from Sichuan province, which is considered to be part of southern China. Studies have reported that in LMV treatment of CHB, resistance primarily took place in HBV with genotypes B and C [19], whereas no difference in rates of resistance has been observed between the 2 genotypes [20,21]. Our results are consistent with these studies, indicating that there isn't a significant difference in the incidence of LMV resistance between genotypes B and genotype C.

It was reported that in China CHB patients with genotype B were younger in age and more likely to be males, compared to those of genotype C (31.4±12.3 *vs.* 33.3±12.2, P=0.01; 16.9% *vs.* 22.4%, P=0.03, for genotypes B and C, respectively) [18]. The mean age of LMV-resistant patients infected with HBV of genotype C (39.1±11.4 years old) were significantly older than that of genotype B (33.7±9.7 years old) (t=–6.55, P<0.01) [22]. However, in our study the mean ages of LMV-resistant patients were not significantly different between genotypes B and C. This discrepancy may be caused by the different sampling in our study, as we chose mainly southern Chinese and the sample size of our study is relatively small. Thus, further investigation is needed to shed light on the age and sex tendency in genotypes B and genotype C.

As the reverse transcriptase lacks a proofreading function, HBV has a high mutation rate. We've detected 6 mutation sites in our study. These sites we detected were also reported in other studies. Our results show that LMV resistance may be highly related to mutations at site rt204 of the YMDD motif. And another mutation position, rt180, which is also located in the YMDD motif and next to rt204 [6], always compensates for the mutation at rt204. These findings agree with the hypothesis that the most frequent mutations are rtL180M and rtM204I/V [23–26]. Otherwise, amino acid change at position rt181 in the RT domain could induce cross-resistance to Lamivudine and Adefovir [27,28], and rt173, rt207 and rt213 sites were known as the common compensatory mutations to confer reduction in susceptibility to Lamivudine [6,29].

According to a previous study, about one-third of CHB patients had rt204 mutation [20].The frequency of rt204 mutation in our study was 42.16% (78/185); 96.30% of the patients with rtM204V also had rtL180M, while 93.48% of the patients with rtM204I did not. This verifies that the rtM204V mutations are always accompanied by rtL180M, while rtM204I mutations are not [30]. The pattern of rtM204I alone was predominantly observed (36.26%), followed by rtM204V + rtL180M (23.08%). The rtM204I alone mutation in genotype B (45.61%) was significantly more frequent than that in genotype C (20.59%) (χ^2=5.77, P<0.05). Xu LJ et al. [22] reported the incidence rate of rtA181V/T mutation in genotype C was significantly higher than that in genotype B. We also identified this mutation; however, the frequencies are basically the same as in genotypes B and C. The clinical significance of this mutation requires further investigation. Other mutations and mixed combinations were also observed (rtM204I+rtV207M/L, rtM204I+rtV173L, rtM204I+rtS213T, rtM204V+rtL180M+rtV173L/M, rtM204V+rtL180M+rtV207M, rtM204I/V+rtL180M+rtS213T), but with the relatively small sample size in this study, it would be inappropriate to draw strong conclusions on the distributions of those genotypes in southern China. We intend to expand the sample size to improve this part of the data in future research.

Conclusions

Our study concludes that LMV has very high resistance incidence, and HBV with genotypes B and C are the main prevalent strains among CHB patients in southern China. There was no significant difference between genotype B and genotype C in the incidence of LMV resistance. The RtM204I pattern in LMV-resistant patients infected with genotype B HBV is much more common than that in patients infected with genotype C HBV. Considering the general prevalence of LMV resistance in southern China, testing before treatment is a rational practice for optimal therapeutic decision-making.

References:

1. WHO. Hepatitis B. *http://www.who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/*. Accessed 16/9/2010
2. WHO. Introduction of Hepatitis B Vaccine into Childhood Immunization Services. Geneva: WHO; 2001
3. Lavanchy D: Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat, 2004; 11: 97–107
4. Colacino JM, Staschke KA: The identification and development of antiviral agents for the treatment of chronic hepatitis B virus infection. Prog Drug Res, 1998; 50: 259–322
5. Fernandez-Soto L, Gonzalez A, Escobar-Jimenez F, Vazquez R, Ocete E, Olea N, Salmeron J: Increased risk of autoimmune thyroid disease in hepatitis C *vs.* hepatitis B before, during, and after discontinuing interferon therapy. Arch Intern Med, 1998; 158: 1445–48
6. Karayiannis P: Hepatitis B virus: old, new and future approaches to antiviral treatment. J Antimicrob Chemother, 2003; 51: 761–85
7. Josefson D: Oral treatment for hepatitis B gets approval in the United States. BMJ, 1998; 317: 1034
8. De Clercq E: Perspectives for the treatment of hepatitis B virus infections. Int J Antimicrob Agents, 1999; 12: 81–95
9. Fung SK, Lok AS: Treatment of chronic hepatitis B: who to treat, what to use, and for how long? Clin Gastroenterol Hepatol, 2004; 2: 839–48
10. Lok ASF, McMahon BJ: Chronic hepatitis B. Hepatology, 2007; 45: 507–39
11. Papadopoulos VP, Chrysagis DN, Protopapas AN et al: Peginterferon alfa-2b as monotherapy or in combination with lamivudine in patients with HBeAg-negative chronic hepatitis B: a randomised study. Med Sci Monit, 2009; 15(2): CR56–61

12. Liaw YF, Chien RN, Yeh CT et al: Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology, 1999; 30: 567–72
13. Bartholomew MM, Jansen RW, Jeffers LJ et al: Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet, 1997; 349: 20–22
14. Aye TT, Bartholomeusz A, Shaw T et al: Hepatitis B virus polymerase mutations during antiviral therapy in a patient following liver transplantation. J Hepatol, 1997; 26: 1148–53
15. Tillmann HL, Trautwein C, Bock T et al: Mutational pattern of hepatitis B virus on sequential therapy with famciclovir and lamivudine in patients with hepatitis B virus reinfection occurring under HBIg immunoglobulin after liver transplantation. Hepatology, 1999; 30: 244–56
16. Malik AH, Lee WM: Chronic hepatitis B virus infection: treatment strategies for the next millennium. Ann Intern Med, 2000; 132: 723–31
17. O'Shea R: Chronic hepatitis B virus infection: issues in treatment. Cleve Clin J Med, 2007; 74: 557–60
18. Zeng G, Wang Z, Wen S et al: Geographic distribution, virologic and clinical characteristics of hepatitis B virus genotypes in China. J Viral Hepat, 2005; 12: 609–17
19. Valsamakis A: Molecular Testing in the Diagnosis and Management of Chronic Hepatitis B. Clin Microbiol Rev, 2007; 20: 426–39
20. Akuta NF, Suzuki M, Kobayashi A et al: The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. J Hepatol, 2003; 38: 315–21
21. Kao JH, Liu CJ, Chen DS: Hepatitis B viral genotypes and lamivudine resistance. J Hepatol, 2002; 36: 303–4
22. Xu LJ, Pan C, Li QG et al: Polymerase region mutations and hepatitis B virus genotypes in chronic hepatitis B patients with poor response to lamivudine. Zhonghua Gan Zang Bing Za Zhi, 2010; 18: 180–83
23. Niesters HG, Honkoop P, Haagsma EB et al: Identification of more than one mutation in the hepatitis B virus polymerase gene arising during prolonged lamivudine treatment. J Infect Dis, 1998; 177: 1382–85
24. Guo S, Fandian Z, Tiancai W: The research progress of the new drug lamivudine against HBV. The New Drug Magazine of China, 2001; 10: 96–99
25. Tipples GA, Ma MM, Fischer KP et al: Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine *in vivo*. Hepatology, 1996; 24: 714–17
26. Ling R, Mutimer D, Ahmed M et al: Selection of mutation in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology, 1996; 24: 711–13
27. Villet S, Pichoud C, Billioud G et al: Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. J Hepatol, 2008; 48: 747–55
28. Yatsuji H, Noguchi C, Hiraga N et al: Emergence of a novel lamivudine-resistant hepatitis B virus variant with a substitution outside the YMDD motif. Antimicrob Agents Chemother, 2006; 50: 3867–74
29. Libbrecht E, Doutreloigne J, Van De Velde H et al: Evolution of Primary and CompensatoryLamivudine ResistanceMutation-s in Chronic Hepatitis B Virus-Infected Patients during Long-Term Lamivudine Treatment, Assessed by a Line Probe Assay. J Clin Microbiol, 2007; 45: 3935–41
30. Enomoto M, Tamori A, Kohmoto MT et al: Mutational Patterns of Hepatitis B Virus Genome and Clinical Outcomes After Emergence of Drug-Resistant Variants During Lamivudine Therapy: Analyses of the Polymerase Gene and Full-Length Sequences. J Med Virol, 2007; 79: 1664–70